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**Bermudez et al.**(10) **Patent No.:** **US 9,109,231 B2**  
(45) **Date of Patent:** **Aug. 18, 2015**(54) **SYNTHETIC INSECTICIDAL PROTEINS**  
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(2013.01); **A01N 63/02** (2013.01); **C07K**  
**14/325** (2013.01)(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Traditionally, the primary method for impacting insect pest populations is the application of broad-spectrum chemical insecticides. However, there is increasing concern about the environmental hazards associated with the production and use of synthetic chemical pesticides. Thus, there is substantial interest in developing alternative pesticides, including biological control of insect pests of agricultural significance using a microbial agent or another species of insect. The present invention provides compositions and methods for such biological control. Modified Cry3 pesticidal polypeptides, polynucleotides encoding such polypeptides, and methods of use are disclosed. The modified polynucleotides provided herein can be used to transform organisms and cells of hosts comprising plant, insects, and microorganisms. The expression of modified polypeptides can provide the host with improved insecticidal activity against one or more insect pathogens.

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	12	63	97	106	117	119	140	152	158	186	206	221	222	230	232	258	292	294
Cry3Aa	I	K	N	N	W	M	Q	V	K	R	I	F	E	K	Q	S	P	E
IP3-1	I	K	N	L	I	I	Q	F	K	R	V	L	E	K	Q	T	S	G
IP3-H1	I	K	N	L	I	I	Q	F	K	R	V	L	E	K	Q	T	S	G
IP3-H2	I	K	N	L	I	I	Q	F	E	E	V	L	E	K	Q	T	S	G
IP3-H3	I	K	N	L	I	I	Q	F	E	E	V	L	S	S	Q	T	S	G
IP3-H4	I	R	D	L	I	I	H	F	K	R	V	L	E	K	Q	T	S	G
IP3-H5	I	R	D	L	I	I	H	F	E	E	V	L	E	K	Q	T	S	G
IP3-H6	I	R	D	L	I	I	H	F	E	E	V	L	S	S	Q	T	S	G
IP3-H7	T	E	N	L	I	I	Q	F	K	R	V	L	E	K	H	T	S	G
IP3-H8	T	E	N	L	I	I	Q	F	E	E	V	L	E	K	H	T	S	G
IP3-H9	T	E	N	L	I	I	Q	F	E	E	V	L	S	S	H	T	S	G

	340	346	384	468	472	491	496	503	531	557	584	589	593	610
Cry3Aa	I	I	F	K	K	A	Q	Q	L	F	K	K	F	I
IP3-1	I	L	K	K	A	Q	Q	Q	L	F	K	K	F	S
IP3-H1	V	L	E	A	L	F	K	T	G	Y	F	L	M	S
IP3-H2	V	L	E	A	L	F	K	T	G	Y	F	L	M	S
IP3-H3	V	L	E	A	L	F	K	T	G	Y	F	L	M	S
IP3-H4	I	L	K	A	Q	Q	K	T	G	Y	L	F	V	S
IP3-H5	I	L	K	A	Q	Q	K	T	G	Y	L	F	V	S
IP3-H6	I	L	K	A	Q	Q	K	T	G	Y	L	F	V	S
IP3-H7	I	L	K	A	Q	Q	E	T	G	H	F	F	M	T
IP3-H8	I	L	K	A	Q	Q	E	T	G	H	F	F	M	T
IP3-H9	I	L	K	A	Q	Q	E	T	G	H	F	F	M	T

1

## SYNTHETIC INSECTICIDAL PROTEINS ACTIVE AGAINST CORN ROOTWORM

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/441,816, filed Feb. 11, 2011, which is hereby incorporated herein in its entirety by reference.

### REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

The official copy of the sequence listing is submitted concurrently with the specification as a text file via EFS-Web, in compliance with the American Standard Code for Information Interchange (ASCII), with a file name of 414541 SEQLIST.txt, a creation date of Feb. 8, 2012 and a size of 167 kilobytes. The sequence listing filed via EFS-Web is part of the specification and is incorporated herein by reference as if set forth in its entirety.

### FIELD OF THE INVENTION

The present invention relates to the fields of plant molecular biology and plant pest control. More particularly, the present invention relates to nucleic acids obtained from *Bacillus thuringiensis* Cry3-family genes that encode  $\delta$ -endotoxins characterized by pesticidal activity against insect pests. Compositions and methods of the invention utilize disclosed nucleic acids, and their encoded modified pesticidal polypeptides, to control pests.

### BACKGROUND OF THE INVENTION

Insect pests are a major factor in the loss of agricultural crops. The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is one of the most devastating corn rootworm species in North America, especially in the midwestern corn-growing areas. A related species, the Northern corn rootworm, *D. barberi* Smith and Lawrence, co-inhabits in much of the range, and is fairly similar in biology to western corn rootworm. A third corn rootworm species, the Southern corn rootworm, *D. undecimpunctata howardi*, causes significant economic damage in other regions.

Corn rootworm larvae can destroy significant percentages of corn if left untreated. In the United States, it is presently estimated that 30 million acres (120,000 km<sup>2</sup>) of corn (out of 80 million grown) are infested with corn rootworms, and that the area is expected to grow over the next 20 years. The United States Department of Agriculture estimates that corn rootworms cause \$1 billion in lost revenue each year, which includes \$800 million in yield loss and \$200 million in cost of treatment for corn growers.

Most of the damage in corn is caused by larval feeding. Newly hatched rootworms locate corn roots in the soil and initially begin feeding on the fine root hairs and burrow into root tips of the corn plant. As larvae grow larger, they feed on and tunnel into primary roots. When rootworms are abundant, larval feeding and deterioration of injured roots by root rot pathogens can result in roots being pruned to the base of the stalk. Severe root injury interferes with the roots' ability to transport water and nutrients into the plant reducing plant growth and resulting in reduced grain production. Severe root injury also may result in lodging of corn plants, making harvest more difficult. Silk feeding by adults can result in pruning of silks at the ear tip, commonly called silk clipping.

2

In field corn, beetle populations are occasionally high enough to cause severe silk clipping during pollen shed, which may interfere with pollination.

Corn rootworms of the genus *Diabrotica* (Coleoptera: Chrysomelidae) are among the most important insect pest of agricultural crops in the United States. For example, the Southern corn rootworm (SCRW), *Diabrotica undecimpunctata howardi* Barber is an economically important pest of corn, cucurbits and peanuts. SCRW *Diabrotica undecimpunctata howardi* Barber, or the spotted cucumber beetle, is widely distributed in North America, occurring in most areas east of the Rocky Mountains, in southern Canada, and in Mexico. It is most abundant and destructive in the southern United States. This insect is multivoltine and overwinters as adults in the southern parts of its range (Branson & Krysan (1981) *Environmental Entomology* 10:826-831). Southern corn rootworms infest the roots of many grass crops and weeds, as well as those of peanuts, alfalfa, and occasionally cucurbits. Annually, 20 to 25 million acres of corn are treated with soil insecticides to protect the crop from corn rootworm larval feeding damage (Fuller et al. (1997) *J Econ Entomol* 90:1332-1340). Soil insecticides applied for the corn rootworm represent one of the major uses of insecticide in the United States. Costs associated with insecticides applied to control larval damage to corn roots and adult damage to corn silks, along with crop losses can approach \$1 billion annually (Metcalf (1986) In M. Kogan [ed.], *Ecological Theory and Integrated Pest Management Practice*. John Wiley & Sons, New York).

Biological control of insect pests of agricultural significance using a microbial agent, such as fungi, bacteria, or another species of insect affords an environmentally friendly and commercially attractive alternative to synthetic chemical pesticides. Generally speaking, the use of biopesticides presents a lower risk of pollution and environmental hazards. Biopesticides also provide greater target specificity than is characteristic of traditional broad-spectrum chemical insecticides. Biopesticides are often less expensive to produce and, thus, improve economic yield for a wide variety of crops.

Microbial insecticides, particularly those obtained from *Bacillus* strains, have played an important role in agriculture as alternatives to chemical pest control. Certain species of microorganisms of the genus *Bacillus* are known to possess pesticidal activity against a broad range of insect pests including *Lepidoptera*, *Diptera*, *Coleoptera*, *Hemiptera*, and others. *Bacillus thuringiensis* and *Bacillus papilliae* are among the most successful biocontrol agents discovered to date. Insect pathogenicity has also been attributed to strains of *B. larvae*, *B. lentimorbus*, *B. sphaericus* (Harwood, ed., ((1989) *Bacillus* (Plenum Press), 306) and *B. cereus* (International Application Publication No. WO 96/10083). Pesticidal activity appears to be concentrated in parasporal crystalline protein inclusions, although pesticidal proteins have also been isolated from the vegetative growth stage of *Bacillus*. Several genes encoding these pesticidal proteins have been isolated and characterized (see, for example, U.S. Pat. Nos. 5,366,892 and 5,840,868).

Recently, agricultural scientists have developed crop plants with enhanced insect resistance by genetically engineering crop plants to produce pesticidal proteins from *Bacillus*. For example, corn and cotton plants have been genetically engineered to produce pesticidal proteins isolated from strains of *B. thuringiensis*. These proteins are known as  $\delta$ -endotoxins or Cry toxins (see, e.g., Aronson (2002) *Cell Mol. Life Sci.* 59(3):417-425; Schnepf et al. (1998) *Microbiol Mol Biol Rev.* 62(3):775-806). These genetically engineered crops are now widely used in American agriculture and have provided the

farmer with an environmentally friendly alternative to traditional insect-control methods. In addition, potatoes genetically engineered to contain pesticidal Cry toxins have been sold to the American farmer. While they have proven to be very successful commercially, these genetically engineered, insect-resistant crop plants provide resistance to only a narrow range of the economically important insect pests.

Accordingly, there remains a need for new Bt toxins with a broader range of insecticidal activity against insect pests including, for example, toxins which are active against a greater variety of insects from the order Coleoptera. In addition, there remains a need for biopesticides having activity against a variety of insect pests and for biopesticides which have improved insecticidal activity.

#### BRIEF SUMMARY OF THE INVENTION

Compositions and methods for protecting a plant from a plant pest are provided. The compositions include novel nucleotide and amino acid sequences for Cry3 pesticidal polypeptides. The presently disclosed polypeptides display enhanced pesticidal activity against plant pests. Polynucleotides comprising nucleotide sequences that encode the pesticidal polypeptides of the invention are further provided. In some embodiments, modified Cry polypeptides of the invention display an improved pesticidal activity relative to the Cry3 native polypeptide (SEQ ID NO:6).

Compositions of the invention include nucleic acid molecules encoding sequences for modified Cry3 pesticidal polypeptides, vectors comprising those nucleic acid molecules, and host cells comprising the vectors. Compositions also include the polypeptide sequences. The nucleotide sequences can be used in DNA constructs or expression cassettes for transformation and expression in organisms, including microorganisms and plants. The nucleotide or amino acid sequences may be synthetic sequences that have been designed for expression in an organism including, but not limited to, a microorganism or a plant. Compositions also comprise transformed bacteria, plants, plant cells, plant tissues, and seeds.

In particular, modified Cry3 proteins are provided having improved activity. Using in silico protein sequence design and DNA shuffling, polypeptides were identified having improved insecticidal activities against coleopteran pests. Methods are provided for producing the modified polypeptides of the invention, and for using such polypeptides for controlling or killing pests of interest, particularly Coleopteran pests.

The compositions and methods of the invention are useful for the production of organisms with pesticide resistance, specifically bacteria and plants. These organisms and compositions derived from them are desirable for agricultural purposes. The compositions of the invention are also useful for generating altered or improved pesticidal proteins.

The following embodiments are encompassed by the present invention:

1. A modified Cry3 pesticidal polypeptide, said polypeptide having one or more amino acid substitutions which confer greater solubility to said polypeptide in a solution having a pH of 5 to 9.

2. The modified Cry3 pesticidal polypeptide of embodiment 1, wherein said one or more substitutions are at a position selected from positions corresponding to positions 12, 63, 97, 106, 117, 119, 140, 152, 158, 186, 206, 221, 222, 230, 232, 258, 292, 294, 340, 346, 384, 468, 472, 491, 496, 503, 557, 584, 589, 593, and 610 of SEQ ID NO:6.

3. A modified Cry3 pesticidal polypeptide, said polypeptide comprising at least one amino acid substitution wherein said substitution makes the polypeptide less basic than the wild type Cry3 polypeptide.

4. The modified Cry3 pesticidal polypeptide of embodiment 3, wherein said substitution comprises replacing a basic amino acid with a neutral or acidic amino acid, replacing a neutral amino acid with an acidic amino acid, or a combination thereof.

5. The modified Cry3 pesticidal polypeptide of embodiment 3, wherein said substitution is at a position selected from positions corresponding to positions 12, 63, 97, 106, 117, 119, 140, 152, 158, 186, 206, 221, 222, 230, 232, 258, 292, 294, 340, 346, 384, 468, 472, 491, 496, 503, 557, 584, 589, 593, and 610 of SEQ ID NO:6.

6. The modified Cry3 pesticidal polypeptide of embodiment 4, wherein said basic amino acid residue comprises a histidine, lysine, or arginine residue and said acidic and/or neutral amino acid residue comprises a glutamic acid or aspartic acid residue.

7. The modified Cry3 pesticidal polypeptide of embodiment 4, wherein said basic amino acid residue comprises a histidine, lysine, or arginine residue and said acidic and/or neutral amino acid residue comprises a serine, threonine, leucine, or valine residue.

8. The modified Cry3 pesticidal polypeptide of any one of embodiments 1-7, wherein said polypeptide comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1, 2, 3, 8, 11, or 14.

9. The modified Cry3 pesticidal polypeptide of embodiment 8, wherein said polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 8, 11, or 14.

10. The modified Cry3 pesticidal polypeptide of any one of embodiments 1-8, wherein said polypeptide further comprises at least one amino acid substitution corresponding to position 152 or 158 of SEQ ID NO: 6, wherein said polypeptide exhibits resistance to protease digestion.

11. A fragment or variant of the modified Cry3 pesticidal polypeptide of any one of embodiments 1-10, wherein said fragment or variant retains pesticidal activity.

12. A polynucleotide having a nucleotide sequence encoding the modified Cry3 pesticidal polypeptide of any one of embodiments 1-10.

13. A DNA construct comprising the polynucleotide of embodiment 12.

14. The DNA construct of embodiment 13, wherein said polynucleotide is operably linked to a promoter that drives expression in a microorganism or a plant.

15. The DNA construct of embodiment 14, wherein said promoter drives expression in a plant.

16. The DNA construct of embodiment 14, wherein said promoter is a root-preferred promoter.

17. A plant comprising the polynucleotide of embodiment 12.

18. A plant comprising the DNA construct of any one of embodiments 13-16.

19. The plant of embodiment 17 or 18, wherein said plant is a monocotyledonous plant.

20. The plant of embodiment 17 or 18, wherein said plant is a dicotyledonous plant.

21. The plant of embodiment 19, wherein said monocotyledonous plant is selected from the group consisting of maize, sugarcane, wheat, rice, barley, sorghum, and rye.

22. The plant of embodiment 21, wherein said monocotyledonous plant is maize.

5

23. A transgenic plant expressing the polypeptide of any one of embodiments 1-10.

24. The plant of embodiment 23, wherein said plant is a monocotyledonous plant.

25. The plant of embodiment 23, wherein said plant is a dicotyledonous plant.

26. The plant of embodiment 24, wherein said monocotyledonous plant is selected from the group consisting of maize, sugarcane, wheat, rice, barley, sorghum, and rye.

27. The plant of embodiment 26, wherein said monocotyledonous plant is maize.

28. A transgenic seed produced by the plant of any one of embodiments 17-27.

29. A method for producing a plant having improved pesticidal activity, said method comprising introducing into said plant a polynucleotide having a nucleotide sequence that encodes a modified Cry3 pesticidal polypeptide, said polypeptide having at least one amino acid substitution, wherein said substitution comprises replacing a basic amino acid residue with an acidic or neutral amino acid, wherein said polynucleotide sequence is operably linked to a promoter sequence that drives expression in a plant cell, and wherein said polypeptide has improved pesticidal activity relative to a wild-type Cry3 polypeptide.

30. The method of embodiment 29, wherein said modified Cry3 pesticidal polypeptide has at least one amino acid substitution at a position selected from the group consisting of positions corresponding to 12, 63, 97, 106, 117, 119, 140, 152, 158, 186, 206, 221, 222, 230, 232, 258, 292, 294, 340, 346, 384, 468, 472, 491, 496, 503, 557, 584, 589, 593, and 610 of SEQ ID NO: 6.

31. The method of embodiment 29, wherein said modified Cry3 pesticidal polypeptide comprises an amino acid substitution that replaces one or more histidine, lysine, or arginine residues with a glutamic acid or aspartic acid residue.

32. The method of embodiment 29, wherein said modified Cry3 pesticidal polypeptide comprises an amino acid substitution that replaces one or more histidine, lysine, or arginine residues with a serine, threonine, leucine, or valine residue.

33. The method of any one of embodiments 29-32, wherein said modified Cry3 pesticidal polypeptide comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 8, 11, or 14.

34. The method of embodiment 33, wherein said modified Cry3 pesticidal polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 8, 11, or 14.

35. The method of any one of embodiments 29-33 wherein said modified Cry3 pesticidal polypeptide comprises at least one amino acid substitution corresponding to positions 152 or 158 of SEQ ID NO: 6 that renders the polypeptide resistant to protease digestion.

36. The method of embodiment 31 or 32, wherein said modified Cry3 pesticidal polypeptide comprises a fragment or variant of SEQ ID NO: 1, 2, 3, 8, 11, or 14, wherein said fragment or variant retains insecticidal activity.

37. A method for controlling an insect pest in an area of cultivation comprising planting the area with the transgenic seed of embodiment 28.

38. A microorganism comprising the polynucleotide of embodiment 12 or the DNA construct of embodiment 13 or 14.

39. A transgenic microorganism that expresses the modified Cry3 pesticidal polypeptide of any one of embodiments 1-10.

40. A pesticidal composition comprising at least one microorganism of embodiment 38 or 39.

6

41. A method for controlling an insect pest in an area of cultivation comprising applying an effective amount of the pesticidal composition of embodiment 40 to an environment of said insect pest.

42. The method of embodiment 41, wherein said insect pest is Coleopteran.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 presents Cry3 pesticidal sequences of the invention with replaced residues and their positions relative to SEQ ID NO: 6 highlighted.

#### DETAILED DESCRIPTION OF THE INVENTION

The invention is drawn to compositions and methods for impacting insect pests, particularly plant pests. Compositions and methods directed to increasing the toxicity of a Cry3 endotoxin are provided. Compositions of the invention include nucleotide and amino acid sequences for novel modified pesticidal polypeptides (e.g., proteins). Modifications to the nucleotide and amino acid sequences include the replacement of basic amino acid residues with acidic or neutral amino acid residues. For example, any one of the basic amino acids (arginine, histidine, and lysine) can be replaced with an acidic amino acid (e.g., aspartic acid and glutamic acid) or a neutral amino acid (e.g., serine, threonine, leucine, and valine).

The Cry3 polypeptides of the invention can be designed by any one of in silico protein design, shuffling of DNA sequences, and directed mutagenesis to change a basic amino acid residue to a neutral or acidic residue. After any modification, the resulting polypeptide is tested for insecticidal activity. Polypeptides having the amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 8, 11, or 14, or variants or fragments thereof are provided. Polynucleotides comprising nucleotide sequences encoding the amino acid sequences set forth in SEQ ID NO: 1, 2, 3, 8, 11, or 14, or variants or fragments thereof are further provided, including but not limited to SEQ ID NOs: 7, 9, 10, 12, 13, and 15, and variants and fragments thereof.

Naturally occurring Cry3Aa protein is insoluble in a low salt buffer at and around neutral pH. These low salt and neutral pH conditions mimic the environment of the corn rootworm (CRW) gut. Accordingly, the activity of Cry3Aa against western corn rootworm (WCRW) and southern corn rootworm (SCRW) is very low. Some amino acid substitutions of the invention confer greater solubility to the modified Cry3Aa polypeptide, relative to the native Cry3Aa polypeptide, in a solution having a pH of about pH 5 to about pH 9. Amino acid substitutions of the invention include those that make the Cry3 polypeptide less basic. Thus, the amino acid substitutions include replacing basic amino acids with neutral or acidic amino acids, as well as the substitution of neutral amino acids to acidic amino acids. These amino acid substitutions can be made to any Cry3 polypeptide, including native (e.g., naturally occurring), mutated, or shuffled sequences. These changes increase the solubility of the Cry3 polypeptide in the rootworm gut. Nucleic acid molecules and nucleotide sequences of the invention can be used to transform any organism to produce the encoded modified Cry3 pesticidal polypeptides. Methods are provided that involve the use of such transformed organisms to impact or control plant pests.

Accordingly, the modified Cry3 polypeptides of the invention comprise Cry3 polypeptides that have been modified to make the polypeptide less basic. That is, the modified Cry3 polypeptides of the invention comprise substitutions that

replace a basic amino acid with a neutral amino acid, or that replace a neutral amino acid with an acidic amino acid. The substitutions are made to replace a basic amino acid with a less basic (e.g., neutral or acidic) amino acid in a wild type (e.g., naturally occurring) Cry3 sequence, particularly, SEQ ID NO:6.

The novel pesticidal polypeptides and nucleotide sequences of the invention can be generated by recombinant engineering, such as through DNA shuffling followed by site-directed mutagenesis. These shuffled polypeptides display increased solubility as well as increased insecticidal activities against WCRW and SCRW that are significantly higher than naturally occurring Cry proteins such as Cry3Aa and Cry8Bb.

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

As used herein, the term “recombinant engineering” or “engineered” connotes the utilization of recombinant DNA technology to introduce (e.g., engineer) a change in the protein structure based on an understanding of the protein’s mechanism of action and a consideration of the amino acids being introduced, deleted, or substituted.

“DNA shuffling” as used herein refers to a method for the in vitro recombination of homologous genes, where the genes to be recombined are randomly fragmented by DNaseI. Methods are well known in the art (e.g., Joern (2003) *Methods in Molecular Biology* 231:85-89). The resulting fragments of the desired size are then purified from an agarose gel and reassembled using numerous cycles of denaturation, annealing, and extension by a polymerase. Recombination occurs when fragments from the different parental DNA strands anneal at a region of high sequence identity. Following this reassembly, full-length chimeras generated through multiple rounds of PCR amplification are cloned into suitable expression cassettes.

As used herein, “in silico protein sequence design” refers to use of computational molecular design and protein sequence analysis strategies for modifying proteins. These approaches employ analysis of the three-dimensional structure of a protein to guide the selection of appropriate amino acid sequences for creating desired properties or functions in the protein of interest. Various academic software packages have been developed for in silico protein design and can be used with the invention, including but not limited to 3D-JIGSAW, CPHModel, GeneSilico, I-TASSER, SWISS-MODEL, Servita Protein Modeling Platform, Abalone, GOR, and Phobius.

The term “Cry3” or “Cry3 family” is used herein to refer to the nucleotide or amino acid sequences of the present invention, which share a high degree of sequence identity or similarity to previously described sequences categorized as Cry3 and/or CryIII. In some embodiments, the sequences of the invention share a high degree of sequence identity or similarity with Cry3Aa1 (SEQ ID NO: 6 or 16), Cry3Aa2 (SEQ ID NO: 17), Cry3Aa3 (SEQ ID NO: 18), Cry3Aa4 (SEQ ID NO: 19), Cry3Aa5 (SEQ ID NO: 20), Cry3Aa6 (SEQ ID NO: 21), Cry3Aa7 (SEQ ID NO: 22), Cry3Aa8 (SEQ ID NO: 23), Cry3Aa9 (SEQ ID NO: 24), Cry3Aa10 (SEQ ID NO: 25), Cry3Aa11 (SEQ ID NO: 26), Cry3Aa12 (SEQ ID NO: 27), Cry3Ba1 (SEQ ID NO: 28), Cry3Ba2 (SEQ ID NO: 29), Cry3Bb1 (SEQ ID NO: 30), Cry3Bb2 (SEQ ID NO: 31), Cry3Bb3 (SEQ ID NO: 32), or Cry3Ca1 (SEQ ID NO: 33). In some of these embodiments, the sequences of the invention share a high degree of sequence identity or similarity with a Cry3Aa protein.

The sequences of the invention are modified from naturally occurring (i.e., found in nature) Cry3 sequences in that they have at least one amino acid substitution and are referred to herein as “modified Cry3” sequences (nucleotide or polypeptide). In some embodiments, the modified Cry3 sequences are modified Cry3Aa sequences.

The modified Cry3 sequences of the invention display high solubility at a neutral pH in low salt solutions. Thus, the sequences of the invention are highly soluble in the gut of the CRW and in solutions having a pH of about pH 5 to about pH 9. In one embodiment, exposed basic amino acid residues are replaced with more acidic amino acid residues providing a negative charge. Likewise, basic amino acids can be replaced with neutral amino acids and neutral amino acids can be replaced with acidic amino acids. By “exposed basic amino acid residues” is intended those amino acids on the surface of the crystal structure of the toxin that are histidine, lysine, or arginine. Thus, the modified Cry3 sequences have one or more substitutions of the amino acids at positions 12, 63, 97, 106, 117, 119, 140, 152, 158, 186, 206, 221, 222, 230, 232, 258, 292, 294, 340, 346, 384, 468, 472, 491, 496, 503, 557, 584, 589, 593, and 610. These positions are relative to the wild type Cry3Aa1 polypeptide set forth in SEQ ID NO:6.

At least one modification can be made at the positions indicated above. Such modifications include the substitution of a basic amino acid for a more acidic or neutral amino acid residue. Such acidic amino acids include aspartic acid and glutamic acid. Acidic amino acids are polar and negatively charged at physiological pH. Examples of such acidic amino acid substitutions can include, without limitation, K63E, N97D, K152E, R158E, K222S, K384E, and K496E.

In another aspect, the present invention provides one or more modifications made to Cry3Aa such that solubility of the protein in the corn rootworm (CRW) gut environment is increased (e.g., enhanced). Native Cry3Aa protein, which does not exhibit insecticidal activity to CRW, is highly insoluble at the pH 5 to pH 9 range in the absence of high salt (Li et al. (1991) *Nature* 353: 815-821). Modified Cry3Aa sequences that demonstrate high CRW activity (see variants listed as IP3-H in Example 1) exhibited high solubility around pH 7 (i.e., pH 5 to pH 9) which is similar to the CRW gut pH.

It is recognized that one or more of the amino acids in a Cry3 polypeptide can be modified. In all instances, changes or modifications can be made at various sites, and the resulting toxin protein tested for activity. Thus, polypeptides encoded by nucleotide sequences comprising mutations will comprise at least one amino acid change or addition relative to the native (i.e., naturally occurring) or background sequence, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 35, 38, 40, 45, 47, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 550, 600, 620, 630, 640, or up to 652 amino acid changes.

In another embodiment, at least one of the protease sensitive residues at positions corresponding to positions 152 and 158 of the naturally occurring protein (e.g., SEQ ID NO: 6) can be modified to render the protein resistant to protease digestion, particularly resistant against trypsin. In one embodiment, the modification includes at least one of the following substitutions: K152E and R158E corresponding to SEQ ID NO: 6. These residues are located at the loop between Alpha 3 and 4. At least one of the residues can be mutated. The mutation(s) render the protein resistant to trypsin digestion in the corn rootworm (CRW) gut. In this manner, changes can be made at this site and the protein tested for digestibility. Any changes that decrease digestibility in the gut at this site are

encompassed herein. Other modifications can include at least one of the following substitutions: K63E, N97D, K152E, R158E, K222S, K384E, and K496E corresponding to SEQ ID NO: 6. As used herein, the terms “proteolytic site” or “cleavage site” refer to an amino acid sequence which confers sensitivity to a class of proteases or a particular protease such that a polypeptide containing the amino acid sequence is digested by the class of proteases or particular protease. A proteolytic site is said to be “sensitive” to the protease(s) that recognize that site. It is appreciated in the art that the efficiency of digestion will vary, and that a decrease in efficiency of digestion can lead to an increase in stability or longevity of the polypeptide in an insect gut. Thus, a proteolytic site may confer sensitivity to more than one protease or class of proteases, but the efficiency of digestion at that site by various proteases may vary. Proteolytic sites include, for example, trypsin sites, chymotrypsin sites, and elastase sites.

In some embodiments, the presently disclosed pesticidal polypeptides or variants or fragments thereof display improved pesticidal activity when compared to the parent polypeptide (e.g., SEQ ID NO: 6, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33) from which it was derived. As used herein, the terms “pesticidal activity” and “insecticidal activity” are used synonymously to refer to activity of an organism or a substance (such as, for example, a protein) that can be measured by, but is not limited to, pest mortality, pest weight loss, pest repellency, and other behavioral and physical changes of a pest after feeding and exposure for an appropriate length of time. In this manner, pesticidal activity impacts at least one measurable parameter of pest fitness. As used herein, “pest” means an organism that interferes with or is harmful to plant development and/or growth. One of skill in the art understands that not all substances or mixtures thereof are equally effective against all pests. Of particular interest herein are pesticidal polypeptides that act as insecticides and thus have biological activity against insect pests. Thus, an organism or substance having pesticidal activity adversely impacts at least one measurable parameter of pest fitness. For example, “pesticidal proteins” are proteins that display pesticidal activity by themselves or in combination with other proteins.

Assays for assessing pesticidal activity are well known in the art. See, e.g., U.S. Pat. Nos. 6,570,005 and 6,339,144. See also Brooke et al. (2001) *Bull. Entomol. Res.* 91:265-272; Chen et al. (2007) *Proc. Natl. Acad. Sci. USA* 104:13901-13906; Crespo et al. (2008) *Appl. Environ. Microb.* 74:130-135; Khambay et al. (2003) *Pest Manag. Sci.* 59:174-182; Liu & Dean (2006) *Protein Eng. Des. Sel.* 19:107-111; Maron et al. (1985) *J. Econ. Entomol.* 78:290-293; Robertson et al., *Pesticide Bioassays with Arthropods* (2<sup>nd</sup> ed., CRC Press 2007); Scott & McKibben (1976) *J. Econ. Entomol.* 71:343-344; Stickman (1985) *Bull. Environ. Contam. Toxicol.* 35:133-142; and Verma et al. (1982) *Water Res.* 16 525-529; as well as U.S. Pat. No. 6,268,181. Examples of insect bioassays include, but are not limited to, pest mortality, pest weight loss, pest repellency, pest attraction, and other behavioral and physical changes of the pest after feeding and exposure to a pesticide or pesticidal polypeptide for an appropriate length of time. General methods include addition of the pesticide, pesticidal polypeptide or an organism having the pesticidal polypeptide to the diet source in an enclosed container. See, e.g., U.S. Pat. Nos. 6,339,144 and 6,570,005. Pesticidal activity can be measured by, but is not limited to, changes in mortality, weight loss, attraction, repellency and other behavioral and physical changes after feeding and exposure for an appropriate length of time.

The preferred developmental stage for testing for pesticidal activity is larvae or immature forms of these above-mentioned insect pests. The insects may be reared in total darkness at from about 20° C. to about 30° C. and from about 30% to about 70% relative humidity. Bioassays may be performed as described in Czapla and Lang (1990) *J. Econ. Entomol.* 83(6):2480-2485. Methods of rearing insect larvae and performing bioassays are well known to one of ordinary skill in the art.

In some embodiments of the invention, the pesticidal gene encodes a *Bacillus thuringiensis* (Bt) toxin or a variant thereof, particularly a homologue of a known Cry toxin. “Bt” or “*Bacillus thuringiensis*” toxin is intended to mean the broader class of toxins found in various strains of *Bacillus thuringiensis*, which includes such toxins as, for example, the vegetative insecticidal proteins and the  $\delta$ -endotoxins. See, for example, Crickmore et al. (1998) *Microbiol. Molec. Biol. Rev.* 62:807-813; and Crickmore et al. (2004) *Bacillus Thuringiensis Toxin Nomenclature* at [lifesci.sussex.ac.uk/Home/Neil\\_Crickmore/Bt](http://lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt), both of which are herein incorporated by reference in their entirety. The vegetative insecticidal proteins (for example, members of the VIP1, VIP2, or VIP3 classes) are secreted insecticidal proteins that undergo proteolytic processing by midgut insect fluids. They have pesticidal activity against a broad spectrum of Lepidopteran insects. See, for example, U.S. Pat. No. 5,877,012, herein incorporated by reference in its entirety. The Bt  $\delta$ -endotoxins are toxic to larvae of a number of insect pests, including members of the Lepidoptera, Diptera, and Coleoptera orders. These insect toxins include, but are not limited to, the Cry toxins, including, for example, Cry1, Cry3, Cry5, Cry8, and Cry9. Of particular interest are pesticidal genes that are homologous to known Cry3 genes. In some instances, polypeptide  $\delta$ -endotoxins of the invention and the nucleotide sequences encoding them will share a high degree of sequence identity or similarity to wild-type (i.e., naturally occurring) Cry3 sequences (e.g., SEQ ID NO: 6, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33). In some of these embodiments, the pesticidal polypeptides of the invention and the nucleotide sequences encoding them will share a high degree of sequence identity or similarity to wild-type Cry3Aa sequences.

The Cry endotoxins initially are produced in an inactive protoxin form, which are proteolytically converted into an active endotoxin through the action of proteases in an insect's gut. Once active, the endotoxin binds to the gut epithelium and forms cation-selective channels that cause cell lysis and subsequent death. See, Carroll et al. (1997) *J. Invertebr. Pathol.* 70:41-49; Oppert (1999) *Arch. Insect Biochem. Phys.* 42:1-12; and Rukmini et al. (2000) *Biochimie* 82:109-116.

Bt Cry proteins have five conserved sequence domains, and three conserved structural domains (see, e.g., de Maagd et al. (2001) *Trends Genetics* 17:193-199). The most amino-terminal conserved structural domain (Domain I) consists of seven alpha helices, with a central hydrophobic helix- $\alpha 5$  encircled by six other amphipathic helices, and is involved in membrane insertion and pore formation. The second conserved structural domain (Domain II) consists of three antiparallel beta-sheets implicated in cell binding, and the most carboxy-terminal conserved structural domain (Domain III) consists of a beta-sandwich. Exposed regions in domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity. The location and properties of these domains are known to those of skill in the art. See, for example, Grochulski et al. (1995) *J. Mol. Biol.* 254:447-464; Morse, Yamamoto, and Stroud (2001) *Structure* 9:409-417; Li et al. (1991) *Nature* 353:815-821;



Galitsky et al. (2001) *Acta Cryst D* 57:1101-1109; Boonserm et al. (2006) *J Bacteriol* 188:3391-3401; Boonserm et al. (2005) *J Mol Biol* 348:363-382; and Guo et al. (2009) *J Struct Biol* 168:259-266.

A polypeptide having “improved pesticidal activity” or “improved pesticidal activity” can refer to a polypeptide exhibiting an increase in activity against a single plant pest or activity against a wider spectrum of plant pests as compared to a reference polypeptide (e.g., naturally occurring Cry3 polypeptide). In some embodiments, the presently disclosed pesticidal polypeptides or variants or fragments thereof display improved pesticidal activity when compared to a naturally occurring Cry 3 polypeptide (e.g., SEQ ID NO: 6, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33). In certain embodiments, the presently disclosed pesticidal polypeptide exhibits a 2-fold to 100-fold greater activity against at least one susceptible insect pest than a naturally occurring polypeptide (e.g., SEQ ID NO: 6, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33), including but not limited to, about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, and 100-fold. A finding of improved or enhanced pesticidal activity requires a demonstration of an increase of pesticidal activity of at least 10%, against the pest target, or at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 100%, 150%, 200%, or 300% or greater increase of pesticidal activity relative to the pesticidal activity of a naturally occurring polypeptide as determined against the same pest.

In certain embodiments, a presently disclosed pesticidal polypeptide or variant or fragment thereof exhibits greater pesticidal activity against a Coleopteran pest when compared to a naturally occurring Cry3 polypeptide. In some of these embodiments, the modified Cry3 polypeptide has greater pesticidal activity against a rootworm, including but not limited to a Southern Corn Rootworm or a Western Corn Rootworm, when compared to a naturally occurring Cry3 polypeptide.

Compositions of the invention include nucleic acids, and fragments and variants thereof that encode pesticidal polypeptides. In particular, the present invention provides for modified nucleotide sequences encoding the amino acid sequences shown in SEQ ID NO:1, 2, 3, 8, 11, or 14. Further provided are modified polypeptides having an amino acid sequence encoded by a nucleic acid molecule described herein.

The invention encompasses isolated or substantially purified polynucleotide or protein compositions. An “isolated” or “purified” polynucleotide or protein, or biologically active portion thereof, is substantially or essentially free from components that normally accompany or interact with the polynucleotide or protein as found in its naturally occurring environment. Thus, an isolated or purified polynucleotide or protein is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Optimally, an “isolated” polynucleotide is free of sequences (optimally protein encoding sequences) that naturally flank the polynucleotide (i.e., sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide is derived. For example, in various embodiments, the isolated polynucleotide can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequence that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide is derived. A protein that is substantially free of cellular material includes preparations

of protein having less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of contaminating protein.

Fragments and variants of the nucleotide and amino acid sequences and the polypeptides encoded thereby are also encompassed by the present invention. As used herein the term “fragment” refers to a portion of a nucleotide sequence of a polynucleotide or a portion of an amino acid sequence of a polypeptide of the invention. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native or corresponding full-length protein and hence possess the relevant biological activity such as pesticidal activity.

Nucleic acids that are fragments of a modified Cry3 family nucleotide sequence comprise at least 16, 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 1,000, 1,200, 1,400, 1,600, 1,800, or 1900 nucleotides, or up to the number of nucleotides present in the modified Cry3 family nucleotide sequences disclosed herein. In particular embodiments, the nucleic acids of the invention disclose fragments derived from (e.g., produced from) a nucleic acid of the invention, wherein the fragment encodes a truncated modified Cry3 family endotoxin characterized by pesticidal activity. The truncated polypeptide encoded by the polynucleotide fragments of the invention are characterized by pesticidal activity that is either equivalent to, or improved, relative to the activity of the corresponding full-length polypeptide encoded by the nucleic acid from which the fragment is derived. In some embodiments, nucleic acid fragments of the invention are truncated at the 3' end of the native or corresponding full-length coding sequence. Nucleic acid fragments may also be truncated at both the 5' and 3' end of the native or corresponding full-length coding sequence.

Furthermore, it is understood that the invention also encompasses polypeptides that are fragments of the exemplary pesticidal proteins of the invention and having lengths of at least about 100, about 200, about 300, about 400, about 500, about 600, about 620, about 630, about 640, about 641, about 642, about 643, or about 644 contiguous amino acids of a pesticidal polypeptide of the invention and retain pesticidal activity.

The term “variant” is used herein to refer to a substantially similar sequence. Variant nucleotide sequences include nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a pesticidal protein of the invention. Generally, variants of a particular nucleotide sequence of the invention will have at least about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or more sequence identity to that particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters. A variant of a nucleotide sequence of the invention may differ from that sequence by as few as 1-15 nucleotides, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 nucleotide.

Variants of a particular nucleotide sequence of the invention (i.e., an exemplary nucleotide sequence) can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant nucleotide sequence and the polypeptide encoded by the reference nucleotide sequence. Percent sequence identity between any two polypeptides can be calculated using sequence alignment programs described elsewhere herein using default parameters. Where any given pair of polynucleotides of the invention is evaluated by comparison of the percent sequence identity shared by the two polypeptides they encode, the percent sequence identity between the two encoded polypeptides is at least about 90%, about 91%, about 92%, about 93%, about

94%, about 95%, about 96%, about 97%, or at least about 98%, about 99% or more sequence identity. A biologically active variant of a protein of the invention may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

As used herein, the term "variant protein" encompasses polypeptides that are derived from the polypeptides of the invention by: deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Accordingly, the term "variant protein" encompasses biologically active fragments that comprise a sufficient number of contiguous amino acid residues to retain the biological activity of the polypeptides of the invention, i.e., to have pesticidal activity.

The modified Cry3 family proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the pesticidal proteins can be prepared by introducing mutations into a synthetic nucleic acid (e.g., DNA molecule). Methods for mutagenesis and nucleic acid alterations are well known in the art. For example, designed changes can be introduced using an oligonucleotide-mediated site-directed mutagenesis technique. See, for example, Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel et al. (1987) *Methods in Enzymol.* 154: 367-382; U.S. Pat. No. 4,873,192; Walker and Gaastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York), and the references cited therein.

The nucleotide sequences of the invention find direct use in methods for impacting pests, particularly insect pests such as pests of the order Coleoptera.

The following terms are used to describe the sequence relationships between two or more polynucleotides or polypeptides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", and, (d) "percentage of sequence identity."

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two polynucleotides. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent sequence identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local alignment algorithm of Smith et al. (1981) *Adv. Appl. Math.* 2:482;

the global alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-local alignment method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, as modified in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, Calif.); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the GCG Wis. Genetics Software Package, Version 10 (available from Accelrys Inc., 9685 Scranton Road, San Diego, Calif., USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins et al. (1988) *Gene* 73:237-244 (1988); Higgins et al. (1989) *CABIOS* 5:151-153; Corpet et al. (1988) *Nucleic Acids Res.* 16:10881-90; Huang et al. (1992) *CABIOS* 8:155-65; and Pearson et al. (1994) *Meth. Mol. Biol.* 24:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) supra. A PAM 120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul et al (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) supra. BLAST nucleotide searches can be performed with the BLASTN program, score=100, wordlength=12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the invention. BLAST protein searches can be performed with the BLASTX program, score=50, wordlength=3, to obtain amino acid sequences homologous to a protein or polypeptide of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul et al. (1997) supra. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See the National Center for Biotechnology Information website on the world wide web at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov). Alignment may also be performed manually by inspection.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the *nwsgapdna.cmp* scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. The term "equivalent program" as used herein refers to any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

GAP uses the algorithm of Needleman and Wunsch (1970) supra, to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It allows for the provision

15

of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the GCG Wis. Genetics Software Package for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 200. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or greater.

GAP presents one member of the family of best alignments. There may be many members of this family, but no other member has a better quality. GAP displays four figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the GCG Wis. Genetics Software Package is BLOSUM62 (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

(c) As used herein, "sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, Calif.).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the

16

number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

As used herein, an amino acid residue of a modified Cry3 polypeptide at the position corresponding to a particular amino acid residue of a naturally occurring Cry3 (e.g., SEQ ID NO: 6) refers to the amino acid residue within the modified Cry3 polypeptide that appears opposite the amino acid residue at a particular position in the naturally occurring Cry3 sequence when the modified Cry3 sequence is aligned with the naturally occurring Cry3 sequence (e.g., SEQ ID NO: 6) for maximum homology using an alignment program, such as one known in the art or one described herein.

The modified Cry3 family sequences of the invention are provided in expression cassettes for expression in the organism of interest. The cassette will include 5' and 3' regulatory sequences operably linked to a modified Cry3 family sequence of the invention. The term "operably linked" as used herein refers to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

Such an expression cassette is provided with a plurality of restriction sites for insertion of the modified Cry3 family sequence to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The expression cassette will include in the 5' to 3' direction of transcription: a transcriptional and translational initiation region (i.e., a promoter), a modified Cry3 family DNA sequence of the invention, and a transcriptional and translational termination region (i.e., termination region) functional in the organism serving as a host. The transcriptional initiation region (i.e., the promoter) may be native, analogous, foreign or heterologous to the host organism and/or to the modified Cry3 family sequence of the invention. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. The term "foreign" as used herein indicates that the promoter is not found in the native organism into which the promoter is introduced. Where the promoter is "foreign" or "heterologous" to the Cry3 family sequence of the invention, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked modified Cry3 family sequence of the invention. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence. Where the promoter is a native or natural sequence, the expression of the operably linked sequence is altered from the wild-type expression, which results in an alteration in phenotype.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous to the promoter, the Cry3 family sequence of interest, the plant host, or any combination thereof).

Convenient termination regions are available from the Ti plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau et al. (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991)

*Cell* 64:671-674; Sanfacon et al. (1991) *Genes Dev.* 5:141-149; Mogen et al. (1990) *Plant Cell* 2:1261-1272; Munroe et al. (1990) *Gene* 91:151-158; Ballas et al. (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi et al. (1987) *Nucleic Acid Res.* 15:9627-9639.

Where appropriate, a nucleic acid may be optimized for increased expression in the host organism. Thus, where the host organism is a plant, the synthetic nucleic acids can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92:1-11 for a discussion of host-preferred codon usage. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray et al. (1989) *Nucleic Acids Res.* 17:477-498). Thus, the maize-preferred codon for a particular amino acid may be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is listed in Table 4 of Murray et al., supra. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Pat. Nos. 5,380, 831, and 5,436,391, and Murray et al. (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other well-characterized sequences that may be deleterious to gene expression. The GC content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. The term "host cell" as used herein refers to a cell which contains a vector and supports the replication and/or expression of the expression vector is intended. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells, or monocotyledonous or dicotyledonous plant cells. An example of a monocotyledonous host cell is a maize host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The expression cassettes may additionally contain 5' leader sequences. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein et al. (1989) *Proc. Natl. Acad. Sci. USA* 86: 6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Gallie et al. (1995) *Gene* 165(2): 233-238), MDMV leader (Maize Dwarf Mosaic Virus), human immunoglobulin heavy-chain binding protein (BiP) (Macejak et al. (1991) *Nature* 353: 90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling et al. (1987) *Nature* 325: 622-625); tobacco mosaic virus leader (TMV) (Gallie et al. (1989) in *Molecular Biology of RNA*, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel et al. (1991) *Virology* 81: 382-385). See also, Della-Cioppa et al. (1987) *Plant Physiol.* 84: 965-968.

In preparing the expression cassette, the various DNA fragments may be manipulated so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, in vitro

mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

A number of promoters can be used in the practice of the invention. The promoters can be selected based on the desired outcome. The nucleic acids can be combined with constitutive, tissue-preferred, inducible, or other promoters for expression in the host organism. Suitable constitutive promoters for use in a plant host cell include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Pat. No. 6,072,050; the core CaMV 35S promoter (Odell et al. (1985) *Nature* 313: 810-812); rice actin (McElroy et al. (1990) *Plant Cell* 2: 163-171); ubiquitin (Christensen et al. (1989) *Plant Mol. Biol.* 12: 619-632 and Christensen et al. (1992) *Plant Mol. Biol.* 18: 675-689); pEMU (Last et al. (1991) *Theor. Appl. Genet.* 81: 581-588); MAS (Velten et al. (1984) *EMBO J.* 3:2723-2730); ALS promoter (U.S. Pat. No. 5,659,026), and the like. Other constitutive promoters include, for example, those discussed in U.S. Pat. Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611.

Depending on the desired outcome, it may be beneficial to express the gene from an inducible promoter. Of particular interest for regulating the expression of the nucleotide sequences of the present invention in plants are wound-inducible promoters. Such wound-inducible promoters, may respond to damage caused by insect feeding, and include potato proteinase inhibitor (pin II) gene (Ryan (1990) *Ann. Rev. Phytopath.* 28: 425-449; Duan et al. (1996) *Nature Biotechnology* 14: 494-498); wun1 and wun2, U.S. Pat. No. 5,428,148; win1 and win2 (Stanford et al. (1989) *Mol. Gen. Genet.* 215: 200-208); systemin (McGurl et al. (1992) *Science* 225: 1570-1573); WIP1 (Rohmeier et al. (1993) *Plant Mol. Biol.* 22: 783-792; Eckelkamp et al. (1993) *FEBS Letters* 323: 73-76); MPI gene (Corderok et al. (1994) *Plant J.* 6(2): 141-150); and the like, herein incorporated by reference.

Additionally, pathogen-inducible promoters may be employed in the methods and nucleotide constructs of the present invention. Such pathogen-inducible promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi et al. (1983) *Neth. J. Plant Pathol.* 89: 245-254; Uknes et al. (1992) *Plant Cell* 4: 645-656; and Van Loon (1985) *Plant Mol. Virol.* 4: 111-116. See also WO 99/43819, herein incorporated by reference.

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau et al. (1987) *Plant Mol. Biol.* 9:335-342; Matton et al. (1989) *Molecular Plant-Microbe Interactions* 2:325-331; Somsisch et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somsisch et al. (1988) *Mol. Gen. Genet.* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977. See also, Chen et al. (1996) *Plant J.* 10:955-966; Zhang et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:2507-2511; Warner et al. (1993) *Plant J.* 3:191-201; Siebertz et al. (1989) *Plant Cell* 1:961-968; U.S. Pat. No. 5,750,386 (nematode-inducible); and the references cited therein. Of particular interest is the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero et al. (1992) *Physiol. Mol. Plant Path.* 41:189-200).

Tissue-preferred promoters can be utilized to target enhanced pesticidal protein expression within a particular plant tissue. Tissue-preferred promoters include those discussed in Yamamoto et al. (1997) *Plant J.* 12(2):255-265; Kawamata et al. (1997) *Plant Cell Physiol.* 38(7):792-803;

Hansen et al. (1997) *Mol. Gen. Genet.* 254(3):337-343; Russell et al. (1997) *Transgenic Res.* 6(2):157-168; Rinehart et al. (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp et al. (1996) *Plant Physiol.* 112(2):525-535; Canevascini et al. (1996) *Plant Physiol.* 112(2):513-524; Yamamoto et al. (1994) *Plant Cell Physiol.* 35(5):773-778; Lam (1994) *Results Probl. Cell Differ.* 20:181-196; Orozco et al. (1993) *Plant Mol. Biol.* 23(6):1129-1138; Matsuoka et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(20):9586-9590; and Guevara-Garcia et al. (1993) *Plant J.* 4(3):495-505. Such promoters can be modified, if necessary, for weak expression.

Root-preferred or root-specific promoters are known and can be selected from the many available from the literature or isolated de novo from various compatible species. See, for example, Hire et al. (1992) *Plant Mol. Biol.* 20(2):207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner (1991) *Plant Cell* 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger et al. (1990) *Plant Mol. Biol.* 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); and Miao et al. (1991) *Plant Cell* 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also Bogusz et al. (1990) *Plant Cell* 2(7):633-641, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomentosa* are described. The promoters of these genes were linked to a  $\beta$ -glucuronidase reporter gene and introduced into both the nonlegume *Nicotiana tabacum* and the legume *Lotus corniculatus*, and in both instances root-specific promoter activity was preserved. Leach and Aoyagi (1991) describe their analysis of the promoters of the highly expressed rolC and rolD root-inducing genes of *Agrobacterium rhizogenes* (see *Plant Science* (Limerick) 79(1):69-76). They concluded that enhancer and tissue-preferred DNA determinants are dissociated in those promoters. Teeri et al. (1989) used gene fusion to lacZ to show that the *Agrobacterium* T-DNA gene encoding octopine synthase is especially active in the epidermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in leaf tissue, an especially desirable combination of characteristics for use with an insecticidal or larvicidal gene (see *EMBO J.* 8(2):343-350). The TR1' gene fused to nptII (neomycin phosphotransferase II) showed similar characteristics. Additional root-preferred promoters include the VtENOD-GRP3 gene promoter (Kuster et al. (1995) *Plant Mol. Biol.* 29(4):759-772); and rolB promoter (Capana et al. (1994) *Plant Mol. Biol.* 25(4):681-691. See also U.S. Pat. Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

Generally, the expression cassette will comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). Additional examples of suitable selectable marker genes include, but are not limited to, genes encoding resistance to chloramphenicol (Herrera Estrella et al. (1983) *EMBO J.* 2:987-992); methotrexate (Herrera Estrella et al. (1983) *Nature* 303:209-213; and Meijer et al. (1991) *Plant Mol. Biol.* 16:807-820); streptomycin (Jones et al. (1987) *Mol. Gen. Genet.* 210:86-91); spectinomycin (Bretagne-Sagnard et al.

(1996) *Transgenic Res.* 5:131-137); bleomycin (Hille et al. (1990) *Plant Mol. Biol.* 7:171-176); sulfonamide (Guerineau et al. (1990) *Plant Mol. Biol.* 15:127-136); bromoxynil (Stalker et al. (1988) *Science* 242:419-423); glyphosate (Shaw et al. (1986) *Science* 233:478-481; and U.S. application Ser. Nos. 10/004,357; and 10/427,692); phosphinothricin (DeBlock et al. (1987) *EMBO J.* 6:2513-2518). See generally, Yarranton (1992) *Curr. Opin. Biotech.* 3: 506-511; Christopherson et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 6314-6318; Yao et al. (1992) *Cell* 71: 63-72; Reznikoff (1992) *Mol. Microbiol.* 6: 2419-2422; Barkley et al. (1980) in *The Operon*, pp. 177-220; Hu et al. (1987) *Cell* 48: 555-566; Brown et al. (1987) *Cell* 49: 603-612; Figge et al. (1988) *Cell* 52: 713-722; Deuschle et al. (1989) *Proc. Natl. Acad. Sci. USA* 86: 5400-5404; Fuerst et al. (1989) *Proc. Natl. Acad. Sci. USA* 86: 2549-2553; Deuschle et al. (1990) *Science* 248: 480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines et al. (1993) *Proc. Natl. Acad. Sci. USA* 90: 1917-1921; Labow et al. (1990) *Mol. Cell. Biol.* 10: 3343-3356; Zambretti et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 3952-3956; Baim et al. (1991) *Proc. Natl. Acad. Sci. USA* 88: 5072-5076; Wyborski et al. (1991) *Nucleic Acids Res.* 19: 4647-4653; Hillenand-Wissman (1989) *Topics Mol. Struc. Biol.* 10: 143-162; Degenkolb et al. (1991) *Antimicrob. Agents Chemother.* 35: 1591-1595; Kleinschmidt et al. (1988) *Biochemistry* 27: 1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 5547-5551; Oliva et al. (1992) *Antimicrob. Agents Chemother.* 36: 913-919; Hlavka et al. (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); and Gill et al. (1988) *Nature* 334: 721-724. Such disclosures are herein incorporated by reference.

The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

The methods of the invention involve introducing a polypeptide or polynucleotide into a plant. "Introducing" is intended to mean presenting to the plant the polynucleotide or polypeptide in such a manner that the sequence gains access to the interior of a cell of the plant. The methods of the invention do not depend on a particular method for introducing a polynucleotide or polypeptide into a plant, only that the polynucleotide or polypeptides gains access to the interior of at least one cell of the plant. Methods for introducing polynucleotide or polypeptides into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

The nucleic acids and nucleotide sequences of the invention may be used to transform any organism to produce the encoded pesticidal proteins. Methods are provided that involve the use of such transformed organisms to impact or control plant pests. The nucleotide sequences of the invention may also be used to transform organelles such as chloroplasts (McBride et al. (1995) *Biotechnology* 13: 362-365; and Kota et al. (1999) *Proc. Natl. Acad. Sci. USA* 96: 1840-1845). The invention therefore provides plants and microorganisms transformed with these novel polynucleotides, and methods involving the use of such nucleic acids, pesticidal compositions, transformed organisms, and products thereof in impacting insect pests.

As used herein, the term "impacting insect pests" refers to effecting changes in insect feeding, growth, and/or behavior at any stage of development, including but not limited to: killing the insect; retarding growth; preventing reproductive capability; antifeedant activity; and the like.

Of particular interest herein are insect pests. As used herein, "insect pest" means an organism in the phylum Arthropoda that interferes with or is harmful to plant development and/or growth, and more specifically means an organism in the class Insecta. The class Insecta can be divided into two groups historically treated as subclasses: (1) wingless insects, known as Apterygota; and (2) winged insects, known as Pterygota. Examples of insect pests include, but are not limited to, insects in the orders Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mallophaga, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera and Thysanura, particularly Coleoptera and Lepidoptera. While technically not insects, arthropods such as arachnids, especially in the order Acari, are included in "insect pest." Insect pests include economically important agronomic, forest, greenhouse, nursery, ornamentals, food and fiber, public and animal health, domestic and commercial structure, household, and stored product pests.

Of particular interest are larvae and adults of the order Coleoptera including weevils from the families Anthribidae, Bruchidae, and Curculionidae including, but not limited to: *Anthonomus grandis* Boheman (boll weevil); *Cylindrocopturus adspersus* LeConte (sunflower stem weevil); *Diaprepes abbreviatus* Linnaeus (Diaprepes root weevil); *Hypera punctata* Fabricius (clover leaf weevil); *Lissorhoptrus oryzophilus* Kuschel (rice water weevil); *Metamasius hemipterus hemipterus* Linnaeus (West Indian cane weevil); *M. hemipterus sericeus* Olivier (silky cane weevil); *Sitophilus granarius* Linnaeus (granary weevil); *S. oryzae* Linnaeus (rice weevil); *Smicronyx fulvus* LeConte (red sunflower seed weevil); *S. sordidus* LeConte (gray sunflower seed weevil); *Sphenophorus maidis* Chittenden (maize billbug); *S. livis* Vaurie (sugarcane weevil); *Rhabdoscelus obscurus* Boisdual (New Guinea sugarcane weevil); flea beetles, cucumber beetles, rootworms, leaf beetles, potato beetles, and leafminers in the family Chrysomelidae including, but not limited to: *Chaetocnema ectypa* Horn (desert corn flea beetle); *C. pulicaria* Melsheimer (corn flea beetle); *Colaspis brunnea* Fabricius (grape colaspis); *Diabrotica barberi* Smith & Lawrence (northern corn rootworm); *D. undecimpunctata howardi* Barber (southern corn rootworm); *D. virgifera virgifera* LeConte (western corn rootworm); *Leptinotarsa decemlineata* Say (Colorado potato beetle); *Oulema melanopus* Linnaeus (cereal leaf beetle); *Phyllotreta cruciferae* Goeze (corn flea beetle); *Zygogramma exclamationis* Fabricius (sunflower beetle); beetles from the family Coccinellidae including, but not limited to: *Epilachna varivestis* Mulsant (Mexican bean beetle); chafers and other beetles from the family Scarabaeidae including, but not limited to: *Antitrogonus parvulus* Britton (Childers cane grub); *Cyclocephala borealis* Arrow (northern masked chafer, white grub); *C. immaculata* Olivier (southern masked chafer, white grub); *Dermolepida albohirtum* Waterhouse (Greyback cane beetle); *Eutheola humilis rugiceps* LeConte (sugarcane beetle); *Lepidiota frenchi* Blackburn (French's cane grub); *Tomarus gibbosus* De Geer (carrot beetle); *T. subtropicus* Blatchley (sugarcane grub); *Phyllotropa crinita* Burmeister (white grub); *P. latifrons* LeConte (June beetle); *Popillia japonica* Newman (Japanese beetle); *Rhizotrogus majalis* Razoumowsky (European chafer); carpet beetles from the family Dermestidae; wireworms from the family Elateridae, *Eleodes* spp., *Melanotus* spp. including *M. communis* Gyllenhal (wireworm); *Conoderus* spp.; *Limoni* spp.; *Agriotes* spp.; *Ctenicera* spp.; *Aeolus* spp.; bark beetles from the family Scolytidae; beetles from the family Tenebrionidae; beetles from the family Cerambycidae such as, but not limited to, *Migdolus fryanus* Westwood

(longhorn beetle); and beetles from the Buprestidae family including, but not limited to, *Aphanisticus cochinchinae seminulum* Obenberger (leaf-mining buprestid beetle).

The invention also encompasses transformed or transgenic plants comprising at least one nucleotide sequence of the invention. In some embodiments, the plant is stably transformed with a nucleotide construct comprising at least one nucleotide sequence of the invention operably linked to a promoter that drives expression in a plant cell. As used herein, the terms "transformed plant" and "transgenic plant" refer to a plant that comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is stably integrated within the genome of a transgenic or transformed plant such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette.

It is to be understood that as used herein the term "transgenic" includes any cell, cell line, callus, tissue, plant part, or plant the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

"Stable transformation" is intended to mean that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof. "Transient transformation" is intended to mean that a polynucleotide is introduced into the plant and does not integrate into the genome of the plant or a polypeptide is introduced into a plant.

Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway et al. (1986) *Biotechniques* 4: 320-334), electroporation (Riggs et al. (1986) *Proc. Natl. Acad. Sci. USA* 83: 5602-5606), *Agrobacterium*-mediated transformation (U.S. Pat. Nos. 5,563, 055 and 5,981,840), direct gene transfer (Paszowski et al. (1984) *EMBO J.* 3: 2717-2722), and ballistic particle acceleration (see, for example, U.S. Pat. Nos. 4,945,050; 5,879, 918; 5,886,244; and 5,932,782; Tomes et al. (1995) in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamburg and Phillips (Springer-Verlag, Berlin); and McCabe et al. (1988) *Biotechnology* 6: 923-926); and *Led* transformation (WO 00/28058). For potato transformation see Tu et al. (1998) *Plant Molecular Biology* 37: 829-838 and Chong et al. (2000) *Transgenic Research* 9: 71-78. Additional transformation procedures can be found in Weissinger et al. (1988) *Ann. Rev. Genet.* 22: 421-477; Sanford et al. (1987) *Particulate Science and Technology* 5: 27-37 (onion); Christou et al. (1988) *Plant Physiol.* 87: 671-674 (soybean); McCabe et al. (1988) *Bio/Technology* 6: 923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P: 175-182 (soybean); Singh et al. (1998) *Theor. Appl. Genet.* 96: 319-324 (soybean); Datta et al. (1990) *Biotechnology* 8: 736-740 (rice); Klein et al. (1988) *Proc. Natl. Acad. Sci. USA* 85: 4305-4309 (maize); Klein et al. (1988) *Biotechnology* 6: 559-563 (maize); U.S. Pat. Nos. 5,240,855; 5,322,783 and 5,324,646; Klein et al. (1988) *Plant Physiol.* 91: 440-444

(maize); Fromm et al. (1990) *Biotechnology* 8: 833-839 (maize); Hooykaas-Van Slogteren et al. (1984) *Nature* (London) 311: 763-764; U.S. Pat. No. 5,736,369 (cereals); Byteler et al. (1987) *Proc. Natl. Acad. Sci. USA* 84: 5345-5349 (Liliaceae); De Wet et al. (1985) in *The Experimental Manipulation of Ovule Tissues*, ed. Chapman et al. (Longman, N.Y.), pp. 197-209 (pollen); Kaeppler et al. (1990) *Plant Cell Reports* 9: 415-418 and Kaeppler et al. (1992) *Theor. Appl. Genet.* 84: 560-566 (whisker-mediated transformation); D'Halluin et al. (1992) *Plant Cell* 4: 1495-1505 (electroporation); Li et al. (1993) *Plant Cell Reports* 12: 250-255 and Christou and Ford (1995) *Annals of Botany* 75: 407-413 (rice); Osjoda et al. (1996) *Nature Biotechnology* 14: 745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

In specific embodiments, the modified Cry3 family sequences of the invention can be provided to a plant using a variety of transient transformation methods. Such transient transformation methods include, but are not limited to, the introduction of the modified Cry3 family protein or variants and fragments thereof directly into the plant or the introduction of the modified Cry3 family transcript into the plant. Such methods include, for example, microinjection or particle bombardment. See, for example, Crossway et al. (1986) *Mol. Gen. Genet.* 202: 179-185; Nomura et al. (1986) *Plant Sci.* 44: 53-58; Hepler et al. (1994) *Proc. Natl. Acad. Sci.* 91: 2176-2180 and Hush et al. (1994) *The Journal of Cell Science* 107: 775-784, all of which are herein incorporated by reference. Alternatively, the modified Cry3 family polynucleotide can be transiently transformed into the plant using techniques known in the art. Such techniques include viral vector system and the precipitation of the polynucleotide in a manner that precludes subsequent release of the DNA. Thus, transcription from the particle-bound DNA can occur, but the frequency with which it is released to become integrated into the genome is greatly reduced. Such methods include the use of particles coated with polyethylimine (PEI; Sigma #P3143).

Methods are known in the art for the targeted insertion of a polynucleotide at a specific location in the plant genome. In one embodiment, the insertion of the polynucleotide at a desired genomic location is achieved using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853, all of which are herein incorporated by reference. Briefly, the polynucleotide of the invention can be contained in transfer cassette flanked by two non-identical recombination sites. The transfer cassette is introduced into a plant have stably incorporated into its genome a target site which is flanked by two non-identical recombination sites that correspond to the sites of the transfer cassette. An appropriate recombinase is provided and the transfer cassette is integrated at the target site. The polynucleotide of interest is thereby integrated at a specific chromosomal position in the plant genome.

The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick et al. (1986) *Plant Cell Reports* 5: 81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive or inducible expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure that expression of the desired phenotypic characteristic has been achieved.

The nucleotide sequences of the invention may be provided to the plant by contacting the plant with a virus or viral nucleic

acids. Generally, such methods involve incorporating the nucleotide construct of interest within a viral DNA or RNA molecule. It is recognized that the recombinant proteins of the invention may be initially synthesized as part of a viral polypeptide, which later may be processed by proteolysis in vivo or in vitro to produce the desired pesticidal protein. It is also recognized that such a viral polypeptide, comprising at least a portion of the amino acid sequence of a pesticidal protein of the invention, may have the desired pesticidal activity. Such viral polypeptides and the nucleotide sequences that encode for them are encompassed by the present invention. Methods for providing plants with nucleotide constructs and producing the encoded proteins in the plants, which involve viral DNA or RNA molecules are known in the art. See, for example, U.S. Pat. Nos. 5,889,191; 5,889,190; 5,866,785; 5,589,367; and 5,316,931; herein incorporated by reference.

As used herein, the term plant also includes plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers, and the like. Grain is intended to mean the mature seed produced by commercial growers for purposes other than growing or reproducing the species. Progeny, variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced polynucleotides.

The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (e.g., *B. napus*, *B. rapa*, *B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables, ornamentals, and conifers.

Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (e.g., *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and chrysanthemum. Conifers that may be employed in practicing the present invention include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus*



*contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*). Plants of the present invention include crop plants (for example, corn, alfalfa, sunflower, *Brassica*, soybean, cotton, safflower, peanut, sorghum, wheat, millet, tobacco, etc.), such as corn and soybean plants.

Turfgrasses include, but are not limited to: annual bluegrass (*Poa annua*); annual ryegrass (*Lolium multiflorum*); Canada bluegrass (*Poa compressa*); Chewings fescue (*Festuca rubra*); colonial bentgrass (*Agrostis tenuis*); creeping bentgrass (*Agrostis palustris*); crested wheatgrass (*Agropyron desertorum*); fairway wheatgrass (*Agropyron cristatum*); hard fescue (*Festuca longifolia*); Kentucky bluegrass (*Poa pratensis*); orchardgrass (*Dactylis glomerata*); perennial ryegrass (*Lolium perenne*); red fescue (*Festuca rubra*); red-top (*Agrostis alba*); rough bluegrass (*Poa trivialis*); sheep fescue (*Festuca ovina*); smooth brome grass (*Bromus inermis*); tall fescue (*Festuca arundinacea*); timothy (*Phleum pratense*); velvet bentgrass (*Agrostis canina*); weeping alkaligrass (*Puccinellia distans*); western wheatgrass (*Agropyron smithii*); Bermuda grass (*Cynodon* spp.); St. Augustine grass (*Stenotaphrum secundatum*); zoysia grass (*Zoysia* spp.); Bahia grass (*Paspalum notatum*); carpet grass (*Axonopus affinis*); centipede grass (*Eremochloa ophiuroides*); kikuyu grass (*Pennisetum clandestinum*); seashore paspalum (*Paspalum vaginatum*); blue gramma (*Bouteloua gracilis*); buffalo grass (*Buchloe dactyloides*); sideoats gramma (*Bouteloua curtipendula*).

Plants of interest include grain plants that provide seeds of interest, oil-seed plants, and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, millet, etc. Oil-seed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, flax, castor, olive etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

In certain embodiments the nucleic acid sequences of the present invention can be stacked with any combination of polynucleotide sequences of interest in order to create plants with a desired phenotype. For example, the polynucleotides of the present invention may be stacked with any other polynucleotides encoding polypeptides having pesticidal and/or insecticidal activity, such as other *Bacillus thuringiensis* toxic proteins (described in U.S. Pat. Nos. 5,366,892; 5,747,450; 5,736,514; 5,723,756; 5,593,881; and Geiser et al., (1986) *Gene* 48:109), pentin (described in U.S. Pat. No. 5,981,722) and the like. The combinations generated can also include multiple copies of any one of the polynucleotides of interest. The polynucleotides of the present invention can also be stacked with any other gene or combination of genes to produce plants with a variety of desired trait combinations including but not limited to traits desirable for animal feed such as high oil genes (e.g., U.S. Pat. No. 6,232,529); balanced amino acids (e.g. hordothionins (U.S. Pat. Nos. 5,990,389; 5,885,801; 5,885,802; and 5,703,049); barley high lysine (Williamson et al. (1987) *Eur. J. Biochem.* 165: 99-106; and WO 98/20122) and high methionine proteins (Pedersen et al. (1986) *J. Biol. Chem.* 261: 6279; Kirihara et al. (1988) *Gene* 71: 359; and Musumura et al. (1989) *Plant Mol. Biol.* 12: 123)); increased digestibility (e.g., modified storage proteins (U.S. application Ser. No. 10/053,410, filed Nov. 7,

2001); and thioredoxins (U.S. application Ser. No. 10/005,429, filed Dec. 3, 2001)), the disclosures of which are herein incorporated by reference.

The invention further provides a method of increasing insect target range by using a pesticidal protein of the invention in combination with at least one other or "second" pesticidal protein. Any pesticidal protein known in the art can be employed in the methods of the present invention. Such pesticidal proteins include, but are not limited to Bt  $\delta$ -endotoxins, protease inhibitors,  $\alpha$ -amylases, and peroxidases.

The polynucleotides of the present invention can also be stacked with traits desirable for disease or herbicide resistance (e.g., fumonisin detoxification genes (U.S. Pat. No. 5,792,931); avirulence and disease resistance genes (Jones et al. (1994) *Science* 266:789; Martin et al. (1993) *Science* 262: 1432; and Mindrinos et al. (1994) *Cell* 78:1089); acetolactate synthase (ALS) mutants that lead to herbicide resistance such as the S4 and/or Hra mutations; inhibitors of glutamine synthase such as phosphinothricin or basta (e.g., bar gene); and glyphosate resistance (EPSPS gene and GAT gene as disclosed in U.S. application Ser. Nos. 10/004,357; and 10/427,692); and traits desirable for processing or process products such as high oil (e.g., U.S. Pat. No. 6,232,529); modified oils (e.g., fatty acid desaturase genes (U.S. Pat. No. 5,952,544; WO 94/11516)); modified starches (e.g., ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (SDBE)); and polymers or bioplastics (e.g., U.S. Pat. No. 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase, and acetoacetyl-CoA reductase (Schubert et al. (1988) *J. Bacteriol.* 170: 5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)), the disclosures of which are herein incorporated by reference. One could also combine the polynucleotides of the present invention with polynucleotides providing agronomic traits such as male sterility (e.g., see U.S. Pat. No. 5,583,210), stalk strength, flowering time, or transformation technology traits such as cell cycle regulation or gene targeting (e.g. WO 99/61619; WO 00/17364; WO 99/25821), the disclosures of which are herein incorporated by reference.

These stacked combinations can be created by any method including but not limited to cross breeding plants by any conventional or TopCross® methodology, or genetic transformation. If the traits are stacked by genetically transforming the plants, the polynucleotide sequences of interest can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of the polynucleotide of interest. This may be combined with any combination of other suppression cassettes or overexpression cassettes to generate the desired combination of traits in the plant. It is further recognized that polynucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853, all of which are herein incorporated by reference.



Compositions of the invention find use in impacting insect pests, and protecting plants, seeds, and plant products in a variety of ways. For example, the compositions can be used in a method that involves placing an effective amount of the pesticidal composition in the environment of the pest by a procedure selected from the group consisting of spraying, dusting, broadcasting, or seed coating.

Before plant propagation material (fruit, tuber, bulb, corm, grains, seed), but especially seed, is sold as a commercial product, it is customarily treated with a protectant coating comprising herbicides, insecticides, fungicides, bactericides, nematocides, molluscicides, or mixtures of several of these preparations, if desired together with further carriers, surfactants, or application-promoting adjuvants customarily employed in the art of formulation to provide protection against damage caused by bacterial, fungal, or animal pests. In order to treat the seed, the protectant coating may be applied to the seeds either by impregnating the tubers or grains with a liquid formulation or by coating them with a combined wet or dry formulation. In addition, in special cases, other methods of application to plants are possible, e.g., treatment directed at the buds or the fruit.

The plant seed of the invention comprising a nucleotide sequence encoding a pesticidal protein of the invention may be treated with a seed protectant coating comprising a seed treatment compound, such as, for example, captan, carboxin, thiram, methalaxyl, pirimiphos-methyl, and others that are commonly used in seed treatment. In one embodiment within the scope of the invention, a seed protectant coating comprising a pesticidal composition of the invention is used alone or in combination with one of the seed protectant coatings customarily used in seed treatment.

It is recognized that the genes encoding the pesticidal proteins can be used to transform insect pathogenic organisms. Such organisms include baculoviruses, fungi, protozoa, bacteria, and nematodes.

The invention further encompasses a microorganism that is transformed with at least one nucleic acid of the invention, with an expression cassette comprising the nucleic acid, or with a vector comprising the expression cassette. In some embodiments, the microorganism is one that multiplies on plants. An embodiment of the invention relates to an encapsulated pesticidal protein which comprises a transformed microorganism capable of expressing at least one pesticidal protein of the invention.

A gene encoding a pesticidal protein of the invention may be introduced via a suitable vector into a microbial host, and said host applied to the environment, or to plants or animals. The term "introduced" in the context of inserting a nucleic acid into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

Microorganism hosts that are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest may be selected. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the pesticidal protein, and desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

Such microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms such as bacteria,

e.g., *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylobacter*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*, fungi, particularly yeast, e.g., *Saccharomyces*, *Cryptococcus*, *Cluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacteria*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioli*, *Alcaligenes entrophus*, *Clavibacter xyli* and *Azotobacter vinlandii* and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluentis*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces rosues*, *S. odoratus*, *Cluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

A number of ways are available for introducing a gene expressing the pesticidal protein into the microorganism host under conditions that allow for stable maintenance and expression of the gene. For example, expression cassettes can be constructed which include the nucleotide constructs of interest operably linked with the transcriptional and translational regulatory signals for expression of the nucleotide constructs, and a nucleotide sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system that is functional in the host, whereby integration or stable maintenance will occur.

Transcriptional and translational regulatory signals include, but are not limited to, promoters, transcriptional initiation start sites, operators, activators, enhancers, other regulatory elements, ribosomal binding sites, an initiation codon, termination signals, and the like. See, for example, U.S. Pat. Nos. 5,039,523 and 4,853,331; EPO 0480762A2; Sambrook et al. (1992) *Molecular Cloning: A Laboratory Manual*, ed. Maniatis et al. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), hereinafter "Sambrook II"; Davis et al., eds. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory Press), Cold Spring Harbor, N.Y.; and the references cited therein.

Suitable host cells, where the pesticidal protein-containing cells will be treated to prolong the activity of the pesticidal proteins in the cell when the treated cell is applied to the environment of the target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells that do not produce substances toxic to higher organisms, such as mammals. However, organisms that produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and gram-positive, include Enterobacteriaceae, such as *Escherichia*, *Erwinia*, *Shigella*, *Salmonella*, and *Proteus*; Bacillaceae; Rhizobiaceae, such as *Rhizobium*; Spirillaceae, such as *Photobacterium*, *Zymomonas*, *Serratia*, *Aeromonas*, *Vibrio*, *Desulfovibrio*, *Spirillum*; Lactobacillaceae; Pseudomonadaceae, such as *Pseudomonas* and *Acetobacter*; Azotobacteraceae and Nitrobacteraceae. Among eukaryotes are fungi, such as *Phycomycetes* and *Ascomycetes*, which includes yeast, such as *Saccharomyces* and *Schizosaccharomyces*; and *Basidiomycetes* yeast, such as *Rhodotorula*, *Aureobasidium*, *Sporobolomyces*, and the like.

Characteristics of particular interest in selecting a host cell for purposes of pesticidal protein production include ease of introducing the pesticidal protein gene into the host, avail-

ability of expression systems, efficiency of expression, stability of the protein in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as *Rhodotorula* spp., *Aureobasidium* spp., *Saccharomyces* spp., and *Sporobolomyces* spp., phylloplane organisms such as *Pseudomonas* spp., *Erwinia* spp., and *Flavobacterium* spp., and other such organisms, including *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Escherichia coli*, *Bacillus* spp., *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus cereus*, and the like.

Genes encoding the pesticidal proteins of the invention can be introduced into microorganisms that multiply on plants (epiphytes) to deliver pesticidal proteins to potential target pests. Epiphytes, for example, can be gram-positive or gram-negative bacteria.

Root-colonizing bacteria, for example, can be isolated from the plant of interest by methods known in the art. Specifically, a *Bacillus cereus* strain that colonizes roots can be isolated from roots of a plant (see, for example, Handelsman et al. (1991) *Appl. Environ. Microbiol.* 56:713-718). Genes encoding the pesticidal proteins of the invention can be introduced into a root-colonizing *Bacillus cereus* by standard methods known in the art.

Genes encoding pesticidal proteins can be introduced, for example, into the root-colonizing *Bacillus* by means of electroporation. Specifically, genes encoding the pesticidal proteins can be cloned into a shuttle vector, for example, pHT3101 (Lerecius et al. (1989) *FEMS Microbiol. Letts.* 60: 211-218). The shuttle vector pHT3101 containing the coding sequence for the particular pesticidal protein gene can, for example, be transformed into the root-colonizing *Bacillus* by means of electroporation (Lerecius et al. (1989) *FEMS Microbiol. Letts.* 60: 211-218).

Expression systems can be designed so that pesticidal proteins are secreted outside the cytoplasm of gram-negative bacteria, such as *E. coli*, for example. Advantages of having pesticidal proteins secreted are: (1) avoidance of potential cytotoxic effects of the pesticidal protein expressed; and (2) improvement in the efficiency of purification of the pesticidal protein, including, but not limited to, increased efficiency in the recovery and purification of the protein per volume cell broth and decreased time and/or costs of recovery and purification per unit protein.

Pesticidal proteins can be made to be secreted in *E. coli*, for example, by fusing an appropriate *E. coli* signal peptide to the amino-terminal end of the pesticidal protein. Signal peptides recognized by *E. coli* can be found in proteins already known to be secreted in *E. coli*, for example the OmpA protein (Ghrayeb et al. (1984) *EMBO J.* 3:2437-2442). OmpA is a major protein of the *E. coli* outer membrane, and thus its signal peptide is thought to be efficient in the translocation process. Also, the OmpA signal peptide does not need to be modified before processing as may be the case for other signal peptides, for example lipoprotein signal peptide (Duffaud et al. (1987) *Meth. Enzymol.* 153: 492).

Pesticidal proteins of the invention can be fermented in a bacterial host and the resulting bacteria processed and used as a microbial spray in the same manner that *Bacillus thuring-*

*iensis* strains have been used as insecticidal sprays. In the case of a pesticidal protein(s) that is secreted from *Bacillus*, the secretion signal is removed or mutated using procedures known in the art. Such mutations and/or deletions prevent secretion of the pesticidal protein(s) into the growth medium during the fermentation process. The pesticidal proteins are retained within the cell, and the cells are then processed to yield the encapsulated pesticidal proteins. Any suitable microorganism can be used for this purpose. *Pseudomonas* has been used to express *Bacillus thuringiensis* endotoxins as encapsulated proteins and the resulting cells processed and sprayed as an insecticide (Gaertner et al. (1993), in: *Advanced Engineered Pesticides*, ed. Kim).

Alternatively, the pesticidal proteins are produced by introducing a heterologous gene into a cellular host. Expression of the heterologous gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. These cells are then treated under conditions that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s). The resulting product retains the toxicity of the toxin. These naturally encapsulated pesticidal proteins may then be formulated in accordance with conventional techniques for application to the environment hosting a target pest, e.g., soil, water, and foliage of plants. See, for example EPA 0192319, and the references cited therein.

In the present invention, a transformed microorganism (which includes whole organisms, cells, spore(s), pesticidal protein(s), pesticidal component(s), pest-impacting component(s), mutant(s), living or dead cells and cell components, including mixtures of living and dead cells and cell components, and including broken cells and cell components) or an isolated pesticidal protein can be formulated with an acceptable carrier into a pesticidal composition(s) that is, for example, a suspension, a solution, an emulsion, a dusting powder, a dispersible granule, a wettable powder, and an emulsifiable concentrate, an aerosol, an impregnated granule, an adjuvant, a coatable paste, and also encapsulations in, for example, polymer substances.

The invention provides pesticidal compositions comprising a transformed microorganism of the invention. In such embodiments, the transformed microorganism is generally present in the pesticidal composition in a pesticidally effective amount, together with a suitable carrier. The invention also encompasses pesticidal compositions comprising an isolated protein of the invention, alone or in combination with a transformed organism of the invention and/or an encapsulated pesticidal protein of the invention, in an insecticidally effective amount, together with a suitable carrier.

Such compositions disclosed above may be obtained by the addition of a surface-active agent, an inert carrier, a preservative, a humectant, a feeding stimulant, an attractant, an encapsulating agent, a binder, an emulsifier, a dye, a UV protectant, a buffer, a flow agent or fertilizers, micronutrient donors, or other preparations that influence plant growth. One or more agrochemicals including, but not limited to, herbicides, insecticides, fungicides, bactericides, nematocides, molluscicides, acaricides, plant growth regulators, harvest aids, and fertilizers, can be combined with carriers, surfactants or adjuvants customarily employed in the art of formulation or other components to facilitate product handling and application for particular target pests. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g., natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders, or fertilizers. The active ingredients of the present invention are normally

applied in the form of compositions and can be applied to the crop area, plant, or seed to be treated. For example, the compositions of the present invention may be applied to grain in preparation for or during storage in a grain bin or silo, etc. The compositions of the present invention may be applied simultaneously or in succession with other compounds. Methods of applying an active ingredient of the present invention or an agrochemical composition of the present invention that contains at least one of the pesticidal proteins produced by the bacterial strains of the present invention include, but are not limited to, foliar application, seed coating, and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

Suitable surface-active agents include, but are not limited to, anionic compounds such as a carboxylate of, for example, a metal; a carboxylate of a long chain fatty acid; an N-acyl-sarcosinate; mono or di-esters of phosphoric acid with fatty alcohol ethoxylates or salts of such esters; fatty alcohol sulfates such as sodium dodecyl sulfate, sodium octadecyl sulfate or sodium cetyl sulfate; ethoxylated fatty alcohol sulfates; ethoxylated alkylphenol sulfates; lignin sulfonates; petroleum sulfonates; alkyl aryl sulfonates such as alkylbenzene sulfonates or lower alkylnaphthalene sulfonates, e.g., butyl-naphthalene sulfonate; salts of sulfonated naphthalene-formaldehyde condensates; salts of sulfonated phenol-formaldehyde condensates; more complex sulfonates such as the amide sulfonates, e.g., the sulfonated condensation product of oleic acid and N-methyl taurine; or the dialkyl sulfo succinates, e.g., the sodium sulfonate of dioctyl succinate. Non-ionic agents include condensation products of fatty acid esters, fatty alcohols, fatty acid amides or fatty-alkyl- or alk-enyl-substituted phenols with ethylene oxide, fatty esters of polyhydric alcohol ethers, e.g., sorbitan fatty acid esters, condensation products of such esters with ethylene oxide, e.g., polyoxyethylene sorbitan fatty acid esters, block copolymers of ethylene oxide and propylene oxide, acetylenic glycols such as 2,4,7,9-tetraethyl-5-decyn-4,7-diol, or ethoxylated acetylenic glycols. Examples of a cationic surface-active agent include, for instance, an aliphatic mono-, di-, or polyamine such as an acetate, naphthenate or oleate; or oxygen-containing amine such as an amine oxide of polyoxyethylene alkylamine; an amide-linked amine prepared by the condensation of a carboxylic acid with a di- or polyamine; or a quaternary ammonium salt.

Examples of inert materials include but are not limited to inorganic minerals such as kaolin, phyllosilicates, carbonates, sulfates, phosphates, or botanical materials such as cork, powdered corncobs, peanut hulls, rice hulls, and walnut shells.

The compositions of the present invention can be in a suitable form for direct application or as a concentrate of primary composition that requires dilution with a suitable quantity of water or other diluant before application. The pesticidal concentration will vary depending upon the nature of the particular formulation, specifically, whether it is a concentrate or to be used directly. The composition contains 1 to 98% of a solid or liquid inert carrier, and 0 to 50% or 0.1 to 50% of a surfactant. These compositions will be administered at the labeled rate for the commercial product, for example, about 0.01 lb-5.0 lb. per acre when in dry form and at about 0.01 pts.-10 pts. per acre when in liquid form.

In a further embodiment, the compositions, as well as the transformed microorganisms and pesticidal proteins, of the invention can be treated prior to formulation to prolong the pesticidal activity when applied to the environment of a target pest as long as the pretreatment is not deleterious to the

pesticidal activity. Such treatment can be by chemical and/or physical means as long as the treatment does not deleteriously affect the properties of the composition(s). Examples of chemical reagents include but are not limited to halogenating agents; aldehydes such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride; alcohols, such as isopropanol and ethanol; and histological fixatives, such as Bouin's fixative and Helly's fixative (see, for example, Humason (1967) *Animal Tissue Techniques* (W.H. Freeman and Co.).

In other embodiments of the invention, it may be advantageous to treat the modified Cry3 family polypeptides with a protease, for example trypsin, to activate the protein prior to application of a pesticidal protein composition of the invention to the environment of the target pest. Methods for the activation of protoxin by a serine protease are well known in the art. See, for example, Cooksey (1968) *Biochem. J.* 6:445-454 and Carroll and Ellar (1989) *Biochem. J.* 261:99-105, the teachings of which are herein incorporated by reference. For example, a suitable activation protocol includes, but is not limited to, combining a polypeptide to be activated, for example a purified modified Cry3 polypeptide (e.g., having the amino acid sequence set forth in SEQ ID NO:2), and trypsin at a 1/100 weight ratio of Cry3 protein/trypsin in 20 mM NaHCO<sub>3</sub>, pH 8 and digesting the sample at 36° C. for 3 hours.

The compositions (including the transformed microorganisms and pesticidal proteins of the invention) can be applied to the environment of an insect pest, a plant, plant seed, plant part, or an area of cultivation by, for example, spraying, atomizing, dusting, scattering, coating or pouring, introducing into or on the soil, introducing into irrigation water, by seed treatment or general application or dusting at the time when the pest has begun to appear or before the appearance of pests as a protective measure. For example, the pesticidal protein and/or transformed microorganisms of the invention may be mixed with grain to protect the grain during storage. It is generally important to obtain good control of pests in the early stages of plant growth, as this is the time when the plant can be most severely damaged. The compositions of the invention can conveniently contain another insecticide if this is thought necessary. In an embodiment of the invention, the composition is applied directly to the soil, at a time of planting, in granular form of a composition of a carrier and dead cells of a *Bacillus* strain or transformed microorganism of the invention. Another embodiment is a granular form of a composition comprising an agrochemical such as, for example, an herbicide, an insecticide, a fertilizer, an inert carrier, and dead cells of a *Bacillus* strain or transformed microorganism of the invention.

The pesticidal composition can be applied to an area of cultivation before or after planting. The area of cultivation can comprise the insect pest or the environmental conditions of the area of cultivation can be conducive to the insect pest (e.g., preferred air temperature, season, soil temperature for growth of the insect pest). As used herein, an "area of cultivation" comprises any region in which one desires to grow a plant. Such areas of cultivations include, but are not limited to, a field in which a plant is cultivated (such as a crop field, a sod field, a tree field, a managed forest, a field for culturing fruits and vegetables, etc), a greenhouse, a growth chamber, etc.

In some embodiments of the invention, the polynucleotide encodes an antibody that binds to an epitope of the modified polypeptide of interest (i.e., modified Cry3 family proteins) when expressed in at least one organism from the group

consisting of plants and microorganism. Such antibodies can be to assay for the expression levels of polypeptides of interest and/or protease activity.

In another embodiment, the binding of the antibody results in increased turnover of the antibody-Cry3 protein complex by cellular quality control mechanisms. The expression of antibodies in plant cells and the inhibition of molecular pathways by expression and binding of antibodies to proteins in plant cells are well known in the art. See, for example, Conrad and Sonnewald (2003) *Nature Biotech.* 21:35-36, incorporated herein by reference.

By "epitope" is intended the part of an antigenic molecule to which an antibody is produced and to which the antibody will bind. Epitopes can comprise linear amino acid residues (i.e., residues within the epitope are arranged sequentially one after another in a linear fashion), nonlinear amino acid residues (referred to herein as "nonlinear epitopes"; these epitopes are not arranged sequentially), or both linear and nonlinear amino acid residues. Typically epitopes are short amino acid sequences, e.g. about five amino acids in length. Systematic techniques for identifying epitopes are known in the art and are described, for example, in U.S. Pat. No. 4,708, 871. Briefly, a set of overlapping oligopeptides derived from the antigen may be synthesized and bound to a solid phase array of pins, with a unique oligopeptide on each pin. The array of pins may comprise a 96-well microtiter plate, permitting one to assay all 96 oligopeptides simultaneously, e.g., for binding to a biomarker-specific monoclonal antibody. Alternatively, phage display peptide library kits (New England BioLabs) are currently commercially available for epitope mapping. Using these methods, the binding affinity for every possible subset of consecutive amino acids may be determined in order to identify the epitope that a given antibody binds. Epitopes may also be identified by inference when epitope length peptide sequences are used to immunize animals from which antibodies are obtained.

Antigen-binding fragments and variants of the antibodies disclosed herein are further provided. Such variants will retain the desired binding properties of the parent antibody. Methods for making antibody fragments and variants are generally available in the art. For example, amino acid sequence variants of an antibody described herein, can be prepared by mutations in the cloned DNA sequence encoding the antibody of interest. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York); Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel et al. (1987) *Methods Enzymol.* 154:367-382; Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, N.Y.); U.S. Pat. No. 4,873,192; and the references cited therein; herein incorporated by reference.

Antibodies having the binding characteristics of an antibody of the invention are also provided. "Binding characteristics" or "binding specificity" when used in reference to an antibody connotes that the antibody recognizes the same or similar antigenic epitope as a comparison antibody. Examples of such antibodies include, for example, an antibody that competes with an antibody of the invention in a competitive binding assay. One of skill in the art could determine whether an antibody competitively interferes with another antibody using standard methods.

In constructing variants of the antibody polypeptide of interest, modifications are made such that variants continue to possess the desired activity, i.e., similar binding specificity to Cry3 proteins. Obviously, any mutations made in the DNA

encoding the variant polypeptide must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See EP Patent Application Publication No. 75,444.

Preferably, variants of an antibody have amino acid sequences that have at least 70% or 75% sequence identity, preferably at least 80% or 85% sequence identity, more preferably at least 90%, 91%, 92%, 93%, 94% or 95% sequence identity to the amino acid sequence for the reference antibody molecule, or to a shorter portion of the reference antibody molecule. More preferably, the molecules share at least 96%, 97%, 98% or 99% sequence identity. For purposes of the present invention, percent sequence identity is determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) *Adv. Appl. Math.* 2:482-489. A variant may, for example, differ from the reference antibody by as few as 1 to 15 amino acid residues, as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

Methods for making antibodies and for selecting appropriate antibodies are known in the art. See, for example, Celis, ed. (in press) *Cell Biology & Laboratory Handbook*, 3rd edition (Academic Press, New York), which is herein incorporated in its entirety by reference. In some embodiments, commercial antibodies directed to Cr proteins may be used to practice the invention.

The following examples are presented by way of illustration, not by way of limitation.

## EXPERIMENTAL

### Example 1

#### IP3-1 Sequence Design

In order to introduce the amino acid sequence diversity to Cry3Aa, a new Cry3-type protein called IP3-1 was designed in silico. In the design process for introducing certain diversities to Cry3Aa, modest changes such as 1→V, L→F, K→H, G→A were made which are expected to induce very small changes in the protein structure. Such structural changes may contribute to higher activity when combined with additional diversity introduced by shuffling.

In order to design IP3-1, all available naturally occurring Cry3 peptide sequences were aligned. The Cry3 sequence was scanned residue by residue to find specific residues that differ among Cry3 proteins. These diversified residues found on Cry3Aa were changed one by one to another residue found in other Cry3 proteins. Each mutation was evaluated structurally by computer to assure structural integrity of the polypeptide. The 3-dimensional conformation of the Cry3Aa derivative was predicted from its amino acid sequence using Homology modeling. Accelrys Discovery Studio™ v 2.0 was used to produce homology models of Cry3 derivatives. The Cry3Aa structure (Protein Data Bank ID 1DLG, Chain A; see Li et al. (1991) *Nature*, 353:815-821) determined by X-ray crystallography was used as reference. Accelrys Discovery Studio recruits MODELER (Sali and Blundell (1993) *J. Mol. Biol.* 234:779-815) to generate model protein structures using conjugate gradient and simulated annealing optimization procedures. The homology model thus built confirms the location within secondary structure element, solvent exposure and detailed orientation of the side-chains of the mutated residue. When significant issues with certain residues were

35

identified during the structural evaluation, such mutations were discarded. After the cycles of mutation and structural evaluation, the final amino acid sequence of IP3-1 was obtained.

The IP3-1 amino acid sequence was back-translated to a nucleotide sequence using *E. coli* codon usage. The IP3-1 nucleotide and amino acid sequences are set forth in SEQ ID NOs: 4 and 5, respectively. The IP3-1 nucleotide sequence was synthesized and cloned in pMAL *E. coli* vector to express the IP3-1 protein as an MBP fusion. The pMAL vector was obtained from New England Biolabs and the synthesized DNA was cloned in the vector, and the MBP-IP3-1 fusion protein was purified according to the manufacturer's recommended method. The fusion protein was expressed in *E. coli* as a soluble protein. However, when the fusion was digested with Factor Xa at pH 8 to isolate the IP3-1 protein from MBP, the IP3-1 protein precipitated. The MBP-free IP3-1 was almost completely insoluble in a wide pH range (pH 5 to pH 9 as tested). The solubility and corn rootworm (CRW) insecticidal activity of this computer-designed IP3-1 appeared to be the same as reported characteristics of naturally occurring Cry3Aa (data not shown).

In order to increase the solubility of IP3-1, several mutations were attempted (FIG. 1). A number of mutants were made as described below, and their solubility and insecticidal activity were assayed. Among those, two sets of mutations, Set 1: K152E-R158E and Set 2: E221S-K232S showed some improved solubility in around pH7. In addition, these mutants showed remarkable activity against WCRW.

IP3-1 DNA was shuffled under mutational conditions as described by Stemmer (1994) *Nature* 370:389-391. Five thousand shuffled variants were selected and screened for insecticidal activity against WCRW. Among those, 3 shuffled variants showed high CRW activity (Table 1). These variants were called IP3-H1, IP3-H4 and IP3-H7 (FIG. 1). Sequencing these variants, the following mutations were found over IP3-1:

IP3-H1: I340V, K384E, Q472L, F589L

IP3-H4: K63R, N97D, Q119H, F584L, I(M)593V

IP3-H7: I12T, K63E, Q232H, K496E, Y557H, S610T

As demonstrated in FIG. 1, each shuffled variant contained at least one mutation that converted a basic amino acid residue to a neutral or acidic residue. IP3-H variants were active against WCRW, SCRW and NCRW, while wild type Cry3Aa and IP3-1 showed low or no activity. From these shuffled CRW active variants, two sets of mutations described above were combined to produce new sequences as follows:

Addition of K152E-R158E mutations produced: H1→H2, H4→H5, and H7→H8

Further addition of E221S-K232S mutations produced: H2→H3, H5→H6, and H8→H9

Purified protein was analyzed by SDS-PAGE to determine the accurate concentration. The protein concentration was adjusted to 2.0 mg/ml and mixed into the insect diet in 96-well plates at 37° C. The diet was made with low temperature melting agarose allowing thorough mixing with IP3 proteins at 37° C. After the diet was solidified at the room temperature, insects (neonate CRW larvae) were infested in each well. The plates were sealed with air permeable plastic film and incubated at 28° C. for 4 days. Insect responses to the IP3 proteins were scored based on feeding inhibition and mortality. EC50 was calculated by Probit analysis and presented in Table 1.

36

TABLE 1

Insecticidal Activity of IP3 Variant Polypeptides			
WCRW		SCRW	
	EC50 ppm		EC50 ppm
IP3-H1	24	IP3-H1	177
IP3-H2	19	IP3-H2	68
IP3-H3	15	IP3-H3	74
IP3-H4	26	IP3-H4	117
IP3-H5	14	IP3-H5	104
IP3-H6	11	IP3-H6	156
IP3-H7	63	IP3-H7	97
IP3-H8	12	IP3-H8	38
IP3-H9	7	IP3-H9	39
IP3-1	500~1000	IP3-1	No Activity

## Example 2

## Generation of IP3 Variant Plant Transformation Constructs

The protein sequences of IP3 variants, H2 (SEQ ID NO: 8), H3 (SEQ ID NO: 1), H5 (SEQ ID NO: 11), H6 (SEQ ID NO: 2), H8 (SEQ ID NO: 14) and H9 (SEQ ID NO: 3) were backtranslated and optimized for expression in maize. The resulting sequences H2 (SEQ ID: 7), H3 (SEQ ID: 9), H5 (SEQ ID: 10), H6 (SEQ ID NO: 12), H8 (SEQ ID NO: 13) and H9 (SEQ ID NO: 15) were synthesized by Genscript USA Corp (Piscataway, N.J.) with BamHI and SnaBI restriction enzyme sites at the 5'- and 3' ends respectively. Each variant was subcloned as a BamHI-SnaBI fragment into a Gateway entry vector containing a plant expression cassette with the maize Ubiquitin1 promoter-5'UTR-intron combination (UBIZM PRO-UBIZM-5UTR-UBIZM INTRON) and the potato PIN II terminator sequence. The resulting plant expression cassette contains the following components operatively linked together in this order; UBIZM PRO-UBIZM-5UTR-UBIZM INTRON, the IP3-variant gene, and the PIN II terminator. The expression cassette is flanked by Gateway attL3 and attL4 recombination sites and this entry vector was used to transfer the expression cassette into an attR3 and attR4 containing binary destination transformation vector. The final plant transformation vector contains the IP3-variant expression cassette upstream of a cassette containing the maize Ubiquitin1 promoter-5'UTR-intron controlling expression of a PAT selectable marker gene with the 35S terminator sequence.

## Example 3

*Agrobacterium*-Mediated Transformation of Maize and Regeneration of Transgenic Plants

For *Agrobacterium*-mediated transformation of maize transformation vectors containing the IP3 variants, the method of Zhao was employed (U.S. Pat. No. 5,981,840, and international patent publication WO98/32326; the contents of each are hereby incorporated by reference in its entirety). Briefly, immature embryos were isolated from maize and the embryos contacted with a suspension of *Agrobacterium* transformed with the IP3 variant plant transformation construct under conditions whereby the bacteria were capable of transferring the gene sequences of the invention to at least one cell of at least one of the immature embryos (step 1: the infection step). In this step, the immature embryos were immersed in an *Agrobacterium* suspension for the initiation

37

of inoculation. The embryos were co-cultured for a time with the *Agrobacterium* (step 2: the co-cultivation step). The immature embryos were cultured on solid medium following the infection step. Following the co-cultivation period, an optional “resting” step was performed. In this resting step, the embryos were incubated in the presence of at least one antibiotic known to inhibit the growth of *Agrobacterium* without the addition of a selective agent for plant transformants (step 3: resting step). The immature embryos were cultured on solid medium with antibiotic, but without a selecting agent, for elimination of *Agrobacterium* and for a resting phase for the infected cells. Next, inoculated embryos were cultured on medium containing a selective agent and growing transformed callus was recovered (step 4: the selection step). The immature embryos were cultured on solid medium with a selective agent resulting in the selective growth of transformed cells. The callus was then regenerated into plants (step 5: the regeneration step), and calli grown on selective medium were cultured on solid medium to regenerate the plants.

#### Example 4

##### Expression and Characterization of IP3 Variants in Transgenic Maize Root Tissue

Tissue samples from V4-V6 roots were placed into 1.2 mL tubes and extracted in 0.6 mL of chilled PBST (Phosphate Buffered Saline plus Tween-20). Following centrifugation, supernatants were removed, diluted, and analyzed by ELISA for IP3 variant expression. The Cry3A ELISA kit used for protein determination was obtained from EnviroLogix, Inc. (Portland, Me.). The ELISA method for the IP3 variant proteins utilized a sequential “sandwich” format to determine the concentration of the protein in sample extracts. Standards (analyzed in triplicate wells) and diluted sample extracts (analyzed in duplicate wells) were incubated in plates pre-coated with an antibody against CRY3A. Following incubation, unbound substances were washed from the plate. A different specific antibody for the respective selected protein, conjugated to the enzyme horseradish peroxidase (HRP), was added to the plate and incubated. Then, unbound substances were washed from the plate, leaving the bound protein “sandwiched” between the antibody coated on the plate and the antibody-HRP conjugate. Detection of the bound antibody-protein complex was accomplished by the addition of substrate, which generated a colored product in the presence of HRP. The reaction was stopped with an acid solution and the optical density (OD) of each well was determined using a plate reader. An average of the results from duplicate wells was used to determine the concentration of the IP3 variant expressed in root tissue in parts per million (PPM) of total soluble protein.

The results of this analysis are shown in Table 2 as an average of each IP3 variant accumulation level in 25 transgenic events. Accumulation was observed with all IP3 variants tested in root tissue. Differences in expression levels between variants can be attributed to multiple factors including the inherent stability and toxicity of each variant in plant tissue, how effective gene optimization was for expression, and differences in cross-reactivity to the CRY3A Ab used for ELISA detection.

38

TABLE 2

Expression of IP3 variants in transgenic maize events.		
	Transgene	Expression in root (ppm)
5	Control	—
	IP3-H2	1571
	IP3-H3	852
	IP3-H5	6493
	IP3-H6	10094
10	IP3-H8	9942
	IP3-H9	9309

#### Example 5

##### Efficacy of Events Expressing IP3 Variants Against Corn Rootworm

Root damage caused by Western Corn Rootworm (WCRW) was investigated with events expressing the IP3 variants. Potted plants at approximately the V4 growth stage were manually infested with approximately 400 WCRW eggs applied into the soil. Root injury scores were taken when adult WCRW begin to emerge from negative control pots. Plants were removed from the pot and gently washed with pressurized water. The root damage was rated using the 0-3 node injury scale (CRWNIS) (Oleson et al. (2005) *J. Econ. Entomol.* 98(1):1-8) and means were calculated for each treatment. Mean root damage ratings from WCRW feeding on events expressing different IP3 variants are shown in Table 3.

TABLE 3

Efficacy of maize expressing individual IP3 variants against WCRW larvae.			
	Variant ID	CRWNIS score*	SD
35	Negative control	2.0	0.30
	IP3-H2	0.12	0.28
	IP3-H3	0.26	0.51
	IP3-H5	0.23	0.34
	IP3-H6	0.44	0.6
	IP3-H8	0.64	0.76
	IP3-H9	0.38	0.58

SD: standard deviation

\*Corn rootworm node injury score (CRWNIS) rating system

Value	Description
0.00	No feeding damage (lowest rating that can be given)
1.00	One node (circle of roots), or the equivalent of an entire node, eaten back to within approximately two inches of the stalk (soil line on the 7th node)
2.00	Two complete nodes eaten
3.00	Three or more nodes eaten (highest rating that can be given)

Damage in between complete nodes eaten is noted as the percentage of the node missing, i.e. 1.50 = 1½ nodes eaten, 0.25 = ¼ of one node eaten, etc.

The results of the rootworm damage assay demonstrate that these IP3 variants provided significantly improved control against WCRW when expressed in transgenic maize. Negative control plants suffered a mean of 2 nodes of root injury compared to node injury scores of within the range of 0.12 to 0.64 for IP3 variant expressing transgenics.

It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “a polypeptide” is understood to represent one or more polypeptides. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

Throughout the specification the word “comprising,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein, the term “about,” when referring to a value is meant to encompass variations of, in some embodiments  $\pm 50\%$ , in some embodiments  $\pm 20\%$ , in some embodiments  $\pm 10\%$ , in some embodiments  $\pm 5\%$ , in some embodiments  $\pm 1\%$ , in some embodiments  $\pm 0.5\%$ , and in some embodiments  $\pm 0.1\%$  from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, when an amount, concentration, or other value or parameter is given as either a range, preferred range, or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of

numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the presently disclosed subject matter be limited to the specific values recited when defining a range.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which the invention pertains. Although any methods and materials similar herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 33

<210> SEQ ID NO 1

<211> LENGTH: 644

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: IP3-H3

<400> SEQUENCE: 1

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 1              5              10              15

Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr
      20              25              30

Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met
      35              40              45

Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp
      50              55              60

Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val
      65              70              75              80

Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu
      85              90              95

Asn Thr Ile Trp Pro Ser Glu Asp Pro Leu Lys Ala Phe Met Glu Gln
      100             105             110

Val Glu Ala Leu Ile Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys
      115             120             125

Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val
      130             135             140

Ser Ala Leu Ser Ser Trp Gln Glu Asn Pro Val Ser Ser Glu Asn Pro
      145             150             155             160

His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His
      165             170             175

Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Gly Tyr Glu Val Leu
      180             185             190

Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
      195             200             205

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-continued

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Lys 210	Asp	Ala	Gln	Ile	Tyr	Gly 215	Glu	Glu	Trp	Gly 220	Tyr	Ser	Ser	Glu	Asp
Ile 225	Ala	Glu	Phe	Tyr	His 230	Arg	Gln	Leu	Lys	Leu 235	Thr	Gln	Glu	Tyr	Thr 240
Asp	His	Cys	Val	Lys 245	Trp	Tyr	Asn	Val	Gly 250	Leu	Asp	Lys	Leu	Arg	Gly 255
Ser	Thr	Tyr	Glu 260	Ser	Trp	Val	Asn	Phe 265	Asn	Arg	Tyr	Arg	Arg	Glu	Met
Thr	Leu	Thr 275	Val	Leu	Asp	Leu	Ile 280	Ala	Leu	Phe	Pro	Leu 285	Tyr	Asp	Val
Arg	Leu 290	Tyr	Ser	Lys	Gly 295	Val	Lys	Thr	Glu	Leu	Thr 300	Arg	Asp	Val	Leu
Thr 305	Asp	Pro	Ile	Val	Gly 310	Val	Asn	Asn	Leu	Arg	Gly 315	Tyr	Gly	Thr	Thr 320
Phe	Ser	Asn	Ile 325	Glu	Asn	Tyr	Ile	Arg	Lys 330	Pro	His	Leu	Phe	Asp	Tyr 335
Leu	His	Arg	Val 340	Gln	Phe	His	Thr	Arg	Leu 345	Gln	Pro	Gly	Tyr	Tyr	Gly 350
Asn	Asp 355	Ser	Phe	Asn	Tyr	Trp	Ser 360	Gly	Asn	Tyr	Val	Ser 365	Thr	Arg	Pro
Ser 370	Ile	Gly	Ser	Asn	Asp	Ile 375	Ile	Thr	Ser	Pro	Phe 380	Tyr	Gly	Asn	Glu
Ser 385	Ser	Glu	Pro	Val	Gln 390	Asn	Leu	Glu	Phe	Asn 395	Gly	Glu	Lys	Val	Tyr 400
Arg	Ala	Val	Ala 405	Asn	Thr	Asn	Leu	Ala	Val 410	Trp	Pro	Ser	Ala	Val	Tyr 415
Ser	Gly	Val	Thr 420	Lys	Val	Glu	Phe	Ser 425	Gln	Tyr	Asn	Asp	Gln	Thr	Asp 430
Glu	Ala 435	Ser	Thr	Gln	Thr	Tyr	Asp 440	Ser	Lys	Arg	Asn	Val 445	Gly	Ala	Val
Ser 450	Trp	Asp	Ser	Ile	Asp	Gln 455	Leu	Pro	Pro	Glu	Thr 460	Thr	Asp	Glu	Pro
Leu 465	Glu	Lys	Ala	Tyr	Ser 470	His	Leu	Leu	Asn	Tyr 475	Val	Met	Cys	Phe	Leu 480
Met	Gln	Gly	Ser 485	Arg	Gly	Thr	Ile	Pro	Val 490	Phe	Thr	Trp	Thr	His	Lys 495
Ser	Val	Asp	Phe 500	Phe	Asn	Thr	Ile	Asp 505	Ser	Lys	Lys	Ile	Thr	Gln	Leu 510
Pro	Leu 515	Val	Lys	Ala	Tyr	Lys	Leu 520	Gln	Ser	Gly	Ala	Ser 525	Val	Val	Ala
Gly 530	Pro	Gly	Phe	Thr	Gly	Gly 535	Asp	Ile	Ile	Gln	Cys 540	Thr	Glu	Asn	Gly
Ser 545	Ala	Ala	Thr	Ile	Tyr 550	Val	Thr	Pro	Asp	Val 555	Ser	Tyr	Ser	Gln	Lys 560
Tyr	Arg	Ala	Arg 565	Ile	His	Tyr	Ala	Ser	Thr 570	Ser	Gln	Ile	Thr	Phe	Thr 575
Leu	Ser	Leu 580	Asp	Gly	Ala	Pro	Phe 585	Asn	Gln	Tyr	Tyr	Leu 590	Asp	Lys	Thr
Met	Asn 595	Lys	Gly	Asp	Thr	Leu 600	Thr	Tyr	Asn	Ser	Phe 605	Asn	Leu	Ala	Ser
Phe 610	Ser	Thr	Pro	Phe	Glu	Leu 615	Ser	Gly	Asn	Asn	Leu 620	Gln	Ile	Gly	Val
Thr	Gly	Leu	Ser	Ala	Gly	Asp	Lys	Val	Tyr	Ile	Asp	Lys	Ile	Glu	Phe



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625	630	635	640
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Ile Pro Val Asn

<210> SEQ ID NO 2  
 <211> LENGTH: 644  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: IP3-H6

<400> SEQUENCE: 2

Met	His	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Thr	Thr	Glu
1			5						10					15	
Asn	Asn	Glu	Val	Pro	Thr	Asn	His	Val	Gln	Tyr	Pro	Leu	Ala	Glu	Thr
	20					25						30			
Pro	Asn	Pro	Thr	Leu	Glu	Asp	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
	35					40					45				
Thr	Ala	Asp	Asn	Asn	Thr	Glu	Ala	Leu	Asp	Ser	Ser	Thr	Thr	Arg	Asp
	50					55				60					
Val	Ile	Gln	Lys	Gly	Ile	Ser	Val	Val	Gly	Asp	Leu	Leu	Gly	Val	Val
65				70					75					80	
Gly	Phe	Pro	Phe	Gly	Gly	Ala	Leu	Val	Ser	Phe	Tyr	Thr	Asn	Phe	Leu
			85					90						95	
Asp	Thr	Ile	Trp	Pro	Ser	Glu	Asp	Pro	Leu	Lys	Ala	Phe	Met	Glu	Gln
		100						105					110		
Val	Glu	Ala	Leu	Ile	Asp	His	Lys	Ile	Ala	Asp	Tyr	Ala	Lys	Asn	Lys
	115					120					125				
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val
	130					135					140				
Ser	Ala	Leu	Ser	Ser	Trp	Gln	Glu	Asn	Pro	Val	Ser	Ser	Glu	Asn	Pro
145				150					155					160	
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His
			165					170						175	
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Gly	Tyr	Glu	Val	Leu
		180					185						190		
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu
	195					200					205				
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	Asp
	210					215					220				
Ile	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr
225				230					235					240	
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly
			245					250						255	
Ser	Thr	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met
	260						265						270		
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val
	275					280					285				
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu
	290					295					300				
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr
305				310					315					320	
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr
			325					330					335		
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Tyr	Gly
		340					345						350		

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Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro
  355                      360                      365

Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys
  370                      375                      380

Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr
  385                      390                      395                      400

Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr
                      405                      410                      415

Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp
                      420                      425                      430

Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val
                      435                      440                      445

Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro
  450                      455                      460

Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu
  465                      470                      475                      480

Met Gln Gly Ser Arg Gly Thr Ile Pro Val Phe Thr Trp Thr His Lys
                      485                      490                      495

Ser Val Asp Phe Phe Asn Thr Ile Asp Ser Lys Lys Ile Thr Gln Leu
                      500                      505                      510

Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala
                      515                      520                      525

Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly
  530                      535                      540

Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys
  545                      550                      555                      560

Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr
                      565                      570                      575

Leu Ser Leu Asp Gly Ala Pro Leu Asn Gln Tyr Tyr Phe Asp Lys Thr
                      580                      585                      590

Val Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser
                      595                      600                      605

Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val
  610                      615                      620

Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe
  625                      630                      635                      640

Ile Pro Val Asn

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<210> SEQ ID NO 3
<211> LENGTH: 644
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IP3-H9

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<400> SEQUENCE: 3

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Met His Pro Asn Asn Arg Ser Glu His Asp Thr Thr Lys Thr Thr Glu
  1                      5                      10                      15

Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr
                      20                      25                      30

Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met
  35                      40                      45

Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Glu Asp
  50                      55                      60

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Val	Ile	Gln	Lys	Gly	Ile	Ser	Val	Val	Gly	Asp	Leu	Leu	Gly	Val	Val	65	70	75	80
Gly	Phe	Pro	Phe	Gly	Gly	Ala	Leu	Val	Ser	Phe	Tyr	Thr	Asn	Phe	Leu	85	90	95	
Asn	Thr	Ile	Trp	Pro	Ser	Glu	Asp	Pro	Leu	Lys	Ala	Phe	Met	Glu	Gln	100	105	110	
Val	Glu	Ala	Leu	Ile	Asp	Gln	Lys	Ile	Ala	Asp	Tyr	Ala	Lys	Asn	Lys	115	120	125	
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val	130	135	140	
Ser	Ala	Leu	Ser	Ser	Trp	Gln	Glu	Asn	Pro	Val	Ser	Ser	Glu	Asn	Pro	145	150	155	160
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His	165	170	175	
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Gly	Tyr	Glu	Val	Leu	180	185	190	
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu	195	200	205	
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	Asp	210	215	220	
Ile	Ala	Glu	Phe	Tyr	His	Arg	His	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr	225	230	235	240
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly	245	250	255	
Ser	Thr	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met	260	265	270	
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val	275	280	285	
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu	290	295	300	
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr	305	310	315	320
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	325	330	335	
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Tyr	Gly	340	345	350	
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro	355	360	365	
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys	370	375	380	
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr	385	390	395	400
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr	405	410	415	
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp	420	425	430	
Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Val	Gly	Ala	Val	435	440	445	
Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro	450	455	460	
Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu	465	470	475	480
Met	Gln	Gly	Ser	Arg	Gly	Thr	Ile	Pro	Val	Phe	Thr	Trp	Thr	His	Glu				

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485	490	495
Ser Val Asp Phe Phe Asn Thr Ile Asp Ser Lys Lys Ile Thr Gln Leu		
500	505	510
Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala		
515	520	525
Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly		
530	535	540
Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser His Ser Gln Lys		
545	550	555
Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr		
565	570	575
Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr		
580	585	590
Met Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser		
595	600	605
Phe Thr Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val		
610	615	620
Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe		
625	630	635
Ile Pro Val Asn		

&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 1935

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Escherichia coli-optimized synthetic sequence.

&lt;400&gt; SEQUENCE: 4

atgcacccta acaacaggtc agagcacgac acgatcaaga caaccgagaa caacgagggtg	60
ccgacgaacc acgtccagta cccactcgcc gaaacaccga accctaccct ggaggacctc	120
aactacaagg agttcctgag gatgacagcg gacaacaaca ccgaagccct cgactctagc	180
acgaccaagg acgtgattca gaagggcacc tctgtggctg gcgacctgct tggcggtgtc	240
gggttcccggt tcggggggcg tctcgtcagc ttctacacga acttcttgaa caccatctgg	300
ccgtccgaag accccctcaa ggcgttcatt gagcagggtg aggcctcat cgaccagaag	360
atcgctgact atgccaagaa caaggctctg gcggagctgc aaggcctgca gaacaacttc	420
gaggactatg tgagcgccct cagctcttgg cagaagaacc cagtcagctc ccgcaaccgg	480
cacagccagg gccgcatcag ggagctgttc agccaggccg agagccactt ccgcaactcc	540
atgccgagct tcgccgtgag cggctacgag gtctctctcc tgactaccta tgcccaggct	600
gcccaacccc acttgcttct cctgaaggac gccagatct acggcgaaga gtggggctac	660
gagaaggagg acatcgccga gttctaccac aggcagctca agctgacgca ggagtacacc	720
gacctgtcgg tgaagtggta caacgtcggc cttgacaagc tgagagggtc tacatacgag	780
agctgggtca acttcaaccg ctataggcgc gagatgacac ttaccgtctc cgacctgac	840
gcgctcttcc ctctgtacga cgtcagactt tactcgaagg gtgtcaagac cgagttgacc	900
agggacgtgc ttaccgaccc aatcgtcggc gtcaacaacc tgcgcggcta cgggaccacc	960
ttcagcaaca tcgagaacta catcaggaag ccgcacctgt tcgactacct gcaccgcaatt	1020
cagttccaca ccaggctgca gcccggtac tacggcaacg acagcttcaa ctactggagc	1080
ggcaactacg tgtctaccag acccagcacc ggctccaacg acatcatcac gtcccgttc	1140

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tacggcaaca agagcagcga gccggtccag aacctggagt tcaacggcga gaaggtctat 1200
cgcgctgtcg ccaacaccaa cctcgcggtc tggccgagcg cgggtgtacag cggcgtcact 1260
aaggtcgagt tcagccagta caacgaccag accgacgagg cgtccacgca gacctacgac 1320
agcaagagga acgttggcgc cgtctcctgg gacagcatcg accagctccc gccagagacc 1380
actgacgagc cacttgagaa ggcttatagc caccagctga actacgtcat gtgcttctct 1440
atgcaaggct ctcgcggcac cattccggtg ttcacctgga cacacaagag cgttgacttc 1500
ttcaacacca tcgacagcaa gaagatcacc cagctccgcg ttgtgaaggc ctacaagctc 1560
cagagcggcg cgagcgtggt cgccgggcct ggtttcacag gcggcgacat cattcagtgt 1620
acgggagaacg gcagcgcggc caccatctac gtcacgcccg acgtgagcta ctcccagaag 1680
tacagagccc gcattcacta cgctccacg agccagatca ccttcacct gagcctcgac 1740
ggcgcgccct tcaaccagta ctacttcgac aagaccatga acaagggcga cacactgacc 1800
tacaacagct tcaacctggc tagcttctct acccattcg agctgagcgg caacaacctg 1860
cagatcggcg tcacaggcct cagcgccggc gacaagggtg acatcgacaa gattgagttc 1920
atcccggtea actga 1935

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&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Escherichia coli-optimized synthetic sequence.

&lt;400&gt; SEQUENCE: 5

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Met His Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu
 1           5           10           15
Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr
 20           25           30
Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met
 35           40           45
Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp
 50           55           60
Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val
 65           70           75           80
Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu
 85           90           95
Asn Thr Ile Trp Pro Ser Glu Asp Pro Leu Lys Ala Phe Met Glu Gln
100          105          110
Val Glu Ala Leu Ile Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys
115          120          125
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val
130          135          140
Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro
145          150          155          160
His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His
165          170          175
Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Gly Tyr Glu Val Leu
180          185          190
Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu
195          200          205
Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp
210          215          220

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Ile	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr	225	230	235	240
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly	245	250	255	
Ser	Thr	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met	260	265	270	
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val	275	280	285	
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu	290	295	300	
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr	305	310	315	320
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	325	330	335	
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Tyr	Gly	340	345	350	
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro	355	360	365	
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys	370	375	380	
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr	385	390	395	400
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr	405	410	415	
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp	420	425	430	
Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Val	Gly	Ala	Val	435	440	445	
Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro	450	455	460	
Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu	465	470	475	480
Met	Gln	Gly	Ser	Arg	Gly	Thr	Ile	Pro	Val	Phe	Thr	Trp	Thr	His	Lys	485	490	495	
Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ser	Lys	Lys	Ile	Thr	Gln	Leu	500	505	510	
Pro	Leu	Val	Lys	Ala	Tyr	Lys	Leu	Gln	Ser	Gly	Ala	Ser	Val	Val	Ala	515	520	525	
Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asp	Ile	Ile	Gln	Cys	Thr	Glu	Asn	Gly	530	535	540	
Ser	Ala	Ala	Thr	Ile	Tyr	Val	Thr	Pro	Asp	Val	Ser	Tyr	Ser	Gln	Lys	545	550	555	560
Tyr	Arg	Ala	Arg	Ile	His	Tyr	Ala	Ser	Thr	Ser	Gln	Ile	Thr	Phe	Thr	565	570	575	
Leu	Ser	Leu	Asp	Gly	Ala	Pro	Phe	Asn	Gln	Tyr	Tyr	Phe	Asp	Lys	Thr	580	585	590	
Met	Asn	Lys	Gly	Asp	Thr	Leu	Thr	Tyr	Asn	Ser	Phe	Asn	Leu	Ala	Ser	595	600	605	
Phe	Ser	Thr	Pro	Phe	Glu	Leu	Ser	Gly	Asn	Asn	Leu	Gln	Ile	Gly	Val	610	615	620	
Thr	Gly	Leu	Ser	Ala	Gly	Asp	Lys	Val	Tyr	Ile	Asp	Lys	Ile	Glu	Phe	625	630	635	640

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Ile Pro Val Asn

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 6

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu
 1             5             10             15

Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr
      20             25             30

Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met
      35             40             45

Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp
 50             55             60

Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val
 65             70             75             80

Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu
      85             90             95

Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln
      100            105            110

Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys
      115            120            125

Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val
      130            135            140

Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro
      145            150            155            160

His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His
      165            170            175

Phe Arg Asn Ser Met Pro Ser Phe Ala Ile Ser Gly Tyr Glu Val Leu
      180            185            190

Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Phe Leu Leu
      195            200            205

Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp
      210            215            220

Ile Ala Glu Phe Tyr Lys Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr
      225            230            235            240

Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly
      245            250            255

Ser Ser Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met
      260            265            270

Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val
      275            280            285

Arg Leu Tyr Pro Lys Glu Val Lys Thr Glu Leu Thr Arg Asp Val Leu
      290            295            300

Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr
      305            310            315            320

Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr
      325            330            335

Leu His Arg Ile Gln Phe His Thr Gln Phe Gln Pro Gly Tyr Tyr Gly
      340            345            350

Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro
      355            360            365

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Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys  
 370 375 380  
 Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr  
 385 390 395 400  
 Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr  
 405 410 415  
 Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp  
 420 425 430  
 Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val  
 435 440 445  
 Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro  
 450 455 460  
 Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu  
 465 470 475 480  
 Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys  
 485 490 495  
 Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu  
 500 505 510  
 Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala  
 515 520 525  
 Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly  
 530 535 540  
 Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys  
 545 550 555 560  
 Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr  
 565 570 575  
 Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr  
 580 585 590  
 Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
 595 600 605  
 Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
 610 615 620  
 Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
 625 630 635 640  
 Ile Pro Val Asn

<210> SEQ ID NO 7  
 <211> LENGTH: 1935  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: IP3-H2  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)...(1935)

<400> SEQUENCE: 7

atg cac cct aac aac agg tca gag cac gac acg atc aag aca acc gag	48
Met His Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu	
1 5 10 15	
aac aac gag gtg ccg acg aac cac gtc cag tac cca ctc gcc gaa aca	96
Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr	
20 25 30	
ccg aac cct acc ctg gag gac ctc aac tac aag gag ttc ctg agg atg	144
Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
aca gcg gac aac aac acc gaa gcc ctc gac tct agc acg acc aag gac	192



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Thr	Ala	Asp	Asn	Asn	Thr	Glu	Ala	Leu	Asp	Ser	Ser	Thr	Thr	Lys	Asp	
50						55					60					
gtg	att	cag	aag	ggc	atc	tct	gtg	gtc	ggc	gac	ctg	ctt	ggc	gtt	gtc	240
Val	Ile	Gln	Lys	Gly	Ile	Ser	Val	Val	Gly	Asp	Leu	Leu	Gly	Val	Val	
65					70					75					80	
ggg	ttc	ccg	ttc	ggc	ggc	gct	ctc	gtc	agc	ttc	tac	acg	aac	ttc	ttg	288
Gly	Phe	Pro	Phe	Gly	Gly	Ala	Leu	Val	Ser	Phe	Tyr	Thr	Asn	Phe	Leu	
				85					90					95		
aac	acc	atc	tgg	ccg	tcc	gaa	gac	ccc	ctc	aag	gcg	ttc	atg	gag	cag	336
Asn	Thr	Ile	Trp	Pro	Ser	Glu	Asp	Pro	Leu	Lys	Ala	Phe	Met	Glu	Gln	
			100					105					110			
gtg	gag	gcc	ctc	atc	gac	cag	aag	atc	gct	gac	tat	gcc	aag	aac	aag	384
Val	Glu	Ala	Leu	Ile	Asp	Gln	Lys	Ile	Ala	Asp	Tyr	Ala	Lys	Asn	Lys	
	115					120					125					
gct	ctg	gcg	gag	ctg	caa	ggc	ctg	cag	aac	aac	ttc	gag	gac	tat	gtg	432
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val	
	130				135						140					
agc	gcc	ctc	agc	tct	tgg	cag	gag	aac	cca	gtc	agc	tcc	gag	aac	ccg	480
Ser	Ala	Leu	Ser	Ser	Trp	Gln	Glu	Asn	Pro	Val	Ser	Ser	Glu	Asn	Pro	
	145				150					155					160	
cac	agc	cag	ggc	cgc	atc	agg	gag	ctg	ttc	agc	cag	gcc	gag	agc	cac	528
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His	
				165					170					175		
ttc	cgc	aac	tcc	atg	ccg	agc	ttc	gcc	gtg	agc	ggc	tac	gag	gtc	ctc	576
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Gly	Tyr	Glu	Val	Leu	
			180					185					190			
ttc	ctg	act	acc	tat	gcc	cag	gct	gcc	aac	acc	cac	ttg	ctt	ctc	ctg	624
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu	
		195				200						205				
aag	gac	gcc	cag	atc	tac	ggc	gaa	gag	tgg	ggc	tac	gag	aag	gag	gac	672
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp	
	210					215				220						
atc	gcc	gag	ttc	tac	cac	agg	cag	ctc	aag	ctg	acg	cag	gag	tac	acc	720
Ile	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr	
	225				230					235					240	
gac	cac	tgc	gtg	aag	tgg	tac	aac	gtc	ggc	ctt	gac	aag	ctg	aga	ggg	768
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly	
				245					250					255		
tct	aca	tac	gag	agc	tgg	gtc	aac	ttc	aac	cgc	tat	agg	cgc	gag	atg	816
Ser	Thr	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met	
			260					265					270			
aca	ctt	acc	gtc	ctc	gac	ctg	atc	gcg	ctc	ttc	cct	ctg	tac	gac	gtc	864
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val	
		275				280						285				
aga	ctt	tac	tcg	aag	ggc	gtc	aag	acc	gag	ttg	acc	agg	gac	gtg	ctt	912
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu	
	290				295					300						
acc	gac	cca	atc	gtc	ggc	gtc	aac	aac	ctg	cgc	ggc	tac	ggg	acc	acc	960
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr	
	305				310					315					320	
ttc	agc	aac	atc	gag	aac	tac	atc	agg	aag	ccg	cac	ctg	ttc	gac	tac	1008
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	
			325						330					335		
ctg	cac	cgc	gtt	cag	ttc	cac	acc	agg	ctg	cag	ccc	ggc	tac	tac	ggc	1056
Leu	His	Arg	Val	Gln	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Tyr	Gly	
			340					345					350			
aac	gac	agc	ttc	aac	tac	tgg	agc	ggc	aac	tac	gtg	tct	acc	aga	ccc	1104
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro	
		355						360					365			

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agc atc ggc tcc aac gac atc atc acg tcc ccg ttc tac ggc aac gag Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Glu 370 375 380	1152
agc agc gag ccg gtc cag aac ctg gag ttc aac ggc gag aag gtc tat Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr 385 390 395 400	1200
cgc gct gtc gcc aac acc aac ctc gcg gtc tgg ccg agc gcg gtg tac Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr 405 410 415	1248
agc ggc gtc act aag gtc gag ttc agc cag tac aac gac cag acc gag Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp 420 425 430	1296
gag gcg tcc acg cag acc tac gac agc aag agg aac gtt ggc gcc gtc Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val 435 440 445	1344
tcc tgg gac agc atc gac cag ctc ccg cca gag acc act gac gag cca Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro 450 455 460	1392
ctt gag aag gct tat agc cac ctg ctg aac tac gtc atg tgc ttc ctc Leu Glu Lys Ala Tyr Ser His Leu Leu Asn Tyr Val Met Cys Phe Leu 465 470 475 480	1440
atg caa ggc tcc cgc ggc acc att ccg gtg ttc acc tgg aca cac aag Met Gln Gly Ser Arg Gly Thr Ile Pro Val Phe Thr Trp Thr His Lys 485 490 495	1488
agc gtt gac ttc ttc aac acc atc gac agc aag aag atc acc cag ctc Ser Val Asp Phe Phe Asn Thr Ile Asp Ser Lys Lys Ile Thr Gln Leu 500 505 510	1536
ccg ctt gtg aag gcc tac aag ctc cag agc ggc gcg agc gtg gtc gcc Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala 515 520 525	1584
ggg cct ggt ttc aca ggc ggc gac atc att cag tgt acg gag aac ggc Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly 530 535 540	1632
agc gcg gcc acc atc tac gtc acg ccg gac gtg agc tac tcc cag aag Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys 545 550 555 560	1680
tac aga gcc cgc atc cac tac gcc tcc acg agc cag atc acc ttc acc Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr 565 570 575	1728
ctg agc ctc gac ggc gcg ccc ttc aac cag tac tac ctc gac aag acc Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Leu Asp Lys Thr 580 585 590	1776
atg aac aag ggc gac aca ctg acc tac aac agc ttc aac ctg gct agc Met Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser 595 600 605	1824
ttc tct acc cca ttc gag ctg agc ggc aac aac ctg cag atc ggc gtc Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val 610 615 620	1872
aca ggc ctc agc gcc ggc gac aag gtg tac atc gac aag att gag ttc Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe 625 630 635 640	1920
atc ccg gtc aac tga Ile Pro Val Asn	1935

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: IP3-H2

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&lt;400&gt; SEQUENCE: 8

Met	His	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Thr	Thr	Glu
1				5					10					15	
Asn	Asn	Glu	Val	Pro	Thr	Asn	His	Val	Gln	Tyr	Pro	Leu	Ala	Glu	Thr
			20					25					30		
Pro	Asn	Pro	Thr	Leu	Glu	Asp	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
		35				40						45			
Thr	Ala	Asp	Asn	Asn	Thr	Glu	Ala	Leu	Asp	Ser	Ser	Thr	Thr	Lys	Asp
	50					55				60					
Val	Ile	Gln	Lys	Gly	Ile	Ser	Val	Val	Gly	Asp	Leu	Leu	Gly	Val	Val
65					70					75				80	
Gly	Phe	Pro	Phe	Gly	Gly	Ala	Leu	Val	Ser	Phe	Tyr	Thr	Asn	Phe	Leu
				85					90					95	
Asn	Thr	Ile	Trp	Pro	Ser	Glu	Asp	Pro	Leu	Lys	Ala	Phe	Met	Glu	Gln
			100					105					110		
Val	Glu	Ala	Leu	Ile	Asp	Gln	Lys	Ile	Ala	Asp	Tyr	Ala	Lys	Asn	Lys
		115					120					125			
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val
	130					135					140				
Ser	Ala	Leu	Ser	Ser	Trp	Gln	Glu	Asn	Pro	Val	Ser	Ser	Glu	Asn	Pro
145					150					155				160	
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His
				165					170					175	
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Gly	Tyr	Glu	Val	Leu
		180						185					190		
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu
	195					200					205				
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp
	210					215					220				
Ile	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr
225					230					235				240	
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly
			245						250					255	
Ser	Thr	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met
		260						265					270		
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val
	275					280					285				
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu
	290					295					300				
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr
305					310					315				320	
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr
			325						330					335	
Leu	His	Arg	Val	Gln	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Tyr	Gly
		340						345					350		
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro
		355						360				365			
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Glu
	370					375					380				
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr
385					390					395				400	
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr
			405						410					415	

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Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp  
 420 425 430  
 Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val  
 435 440 445  
 Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro  
 450 455 460  
 Leu Glu Lys Ala Tyr Ser His Leu Leu Asn Tyr Val Met Cys Phe Leu  
 465 470 475 480  
 Met Gln Gly Ser Arg Gly Thr Ile Pro Val Phe Thr Trp Thr His Lys  
 485 490 495  
 Ser Val Asp Phe Phe Asn Thr Ile Asp Ser Lys Lys Ile Thr Gln Leu  
 500 505 510  
 Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala  
 515 520 525  
 Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly  
 530 535 540  
 Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys  
 545 550 555 560  
 Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr  
 565 570 575  
 Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Leu Asp Lys Thr  
 580 585 590  
 Met Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
 595 600 605  
 Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
 610 615 620  
 Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
 625 630 635 640  
 Ile Pro Val Asn

<210> SEQ ID NO 9  
 <211> LENGTH: 1935  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: IP3-H3

<400> SEQUENCE: 9

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 ccgacgaacc acgtccagta cccactcgcc gaaacaccga accctaccct ggaggacctc 120  
 aactacaagg agttcctgag gatgacagcg gacaacaaca ccgaagccct cgactctagc 180  
 acgaccaagg acgtgattca gaagggcatc tctgtggtcg gcgacctgct tggcggttgc 240  
 gggttcccg tccggcggcg tctcgtcagc ttctacacga acttcttgaa caccatctgg 300  
 ccgtccgaag accccctcaa ggcgttcacg gagcaggtgg aggcctcat cgaccagaag 360  
 atcgctgact atgccaagaa caaggctctg gcggagctgc aaggcctgca gaacaacttc 420  
 gaggactatg tgagcgccct cagctcttgg caggagaacc cagtcagctc cgagaacccg 480  
 cacagccagg gccgcatcag ggagctgttc agccaggccg agagccactt ccgcaactcc 540  
 atgccgagct tcgccgtgag cggctacgag gtcctcttcc tgactaccta tgcccaggct 600  
 gccaacaccc acttgcttct cctgaaggac gccagatct acggcgaaga gtggggctac 660  
 agctctgagg acatcgccga gttctaccac aggcagctca agctgacgca ggagtacacc 720

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gaccactgcg tgaagtggta caacgtcggc cttgacaagc tgagagggtc tacatacgag	780
agctgggtca acttcaaccg ctataggcgc gagatgacac ttaccgtcct cgacctgac	840
gcgctcttcc ctctgtacga cgtcagactt tactcgaagg gtgtcaagac cgagttgacc	900
agggacgtgc ttaccgaccc aatcgtcggc gtcaacaacc tgcgcggcta cgggaccacc	960
ttcagcaaca tcgagaacta catcaggaag ccgcacctgt tcgactacct gcaccgcgtt	1020
cagttccaca ccaggctgca gcccggttac tacggcaacg acagcttcaa ctactggagc	1080
ggcaactacg tgtctaccag acccagcacc ggctccaacg acatcatcac gtccccgttc	1140
tacggcaacg agagcagcga gccgggtccag aacctggagt tcaacggcga gaaggtctat	1200
cgcgctgtcg ccaacaccaa cctcgcggtc tggcgcgagc cggtgtacag cggcgtaact	1260
aaggtcagat tcagccagta caacgaccag accgacgagg cgtccacgca gacctacgac	1320
agcaagagga acgttgggcg cgtctcctgg gacagcatcg accagctccc gccagagacc	1380
actgacgagc cacttgagaa ggcttatagc cacctgctga actacgtcat gtgcttcttc	1440
atgcaaggct cccgcggcac cattccggtg ttcacctgga cacacaagag cgttgacttc	1500
ttcaacacca tcgacagcaa gaagatcacc cagctcccgcc ttgtgaaggc ctacaagctc	1560
cagagcggcg cgagcgtggt cgccgggctt ggtttcacag gcggcgacat cattcagtgt	1620
acggagaaac gcagcgcggc caccatctac gtcacgcccg acgtgagcta ctcccagaag	1680
tacagagccc gcattccacta cgctccacg agccagatca ccttcacctt gagcctcgac	1740
ggcgcgcctt tcaaccagta ctacctgac aagaccatga acaaggcgca cacactgacc	1800
tacaacagct tcaacctggc tagcttctct accccattcg agctgagcgg caacaacctg	1860
cagatcggcg tcacaggcct cagcgcgggc gacaaggtgt acatcgacaa gattgagttc	1920
atcccggtca actga	1935

<210> SEQ ID NO 10  
 <211> LENGTH: 1935  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: IP3-H5  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1) ... (1935)

<400> SEQUENCE: 10

atg cac cct aac aac agg tca gag cac gac acg atc aag aca acc gag	48
Met His Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu	
1 5 10 15	
aac aac gag gtg ccg acg aac cac gtc cag tac cca ctc gcc gaa aca	96
Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr	
20 25 30	
ccg aac cct acc ctg gag gac ctc aac tac aag gag ttc ctg agg atg	144
Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
aca gcg gac aac aac acc gaa gcc ctc gac tct agc acg acc agg gac	192
Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Arg Asp	
50 55 60	
gtg att cag aag ggc atc tct gtg gtc ggc gac ctg ctt ggc gtt gtc	240
Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val	
65 70 75 80	
ggg ttc ccg ttc ggc ggc gct ctc gtc agc ttc tac acg aac ttc ttg	288
Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu	
85 90 95	

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gac acc atc tgg ccg tcc gaa gac ccc ctc aag gcg ttc atg gag cag Asp Thr Ile Trp Pro Ser Glu Asp Pro Leu Lys Ala Phe Met Glu Gln 100 105 110	336
gtg gag gcc ctc atc gac cat aag atc gct gac tat gcc aag aac aag Val Glu Ala Leu Ile Asp His Lys Ile Ala Asp Tyr Ala Lys Asn Lys 115 120 125	384
gct ctg gcg gag ctg caa ggc ctg cag aac aac ttc gag gac tat gtg Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val 130 135 140	432
agc gcc ctc agc tct tgg cag gag aac cca gtc agc tcc gag aac ccg Ser Ala Leu Ser Ser Trp Gln Glu Asn Pro Val Ser Ser Glu Asn Pro 145 150 155 160	480
cac agc cag gcc cgc atc agg gag ctg ttc agc cag gcc gag agc cac His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His 165 170 175	528
ttc cgc aac tcc atg ccg agc ttc gcc gtg agc gcc tac gag gtc ctc Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Gly Tyr Glu Val Leu 180 185 190	576
ttc ctg act acc tat gcc cag gct gcc aac acc cac ttg ctt ctc ctg Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu 195 200 205	624
aag gac gcc cag atc tac ggc gaa gag tgg gcc tac gag aag gag gac Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp 210 215 220	672
atc gcc gag ttc tac cac agg cag ctc aag ctg acg cag gag tac acc Ile Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr 225 230 235 240	720
gac cac tgc gtg aag tgg tac aac gtc gcc ctt gac aag ctg aga ggg Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly 245 250 255	768
tct aca tac gag agc tgg gtc aac ttc aac cgc tat agg cgc gag atg Ser Thr Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met 260 265 270	816
aca ctt acc gtc ctc gac ctg atc gcg ctc ttc cct ctg tac gac gtc Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val 275 280 285	864
aga ctt tac tcg aag ggt gtc aag acc gag ttg acc agg gac gtg ctt Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Val Leu 290 295 300	912
acc gac cca atc gtc ggc gtc aac aac ctg cgc gcc tac ggg acc acc Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr 305 310 315 320	960
ttc agc aac atc gag aac tac atc agg aag ccg cac ctg ttc gac tac Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr 325 330 335	1008
ctg cac cgc att cag ttc cac acc agg ctg cag ccc gcc tac tac ggc Leu His Arg Ile Gln Phe His Thr Arg Leu Gln Pro Gly Tyr Tyr Gly 340 345 350	1056
aac gac agc ttc aac tac tgg agc ggc aac tac gtg tct acc aga ccc Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro 355 360 365	1104
agc atc gcc tcc aac gac atc atc acg tcc ccg ttc tac gcc aac aag Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys 370 375 380	1152
agc agc gag ccg gtc cag aac ctg gag ttc aac gcc gag aag gtc tat Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr 385 390 395 400	1200
cgc gct gtc gcc aac acc aac ctc gcg gtc tgg ccg agc gcg gtg tac Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr 405 410 415	1248

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agc ggc gtc act aag gtc gag ttc agc cag tac aac gac cag acc gac      1296
Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp
          420          425          430

gag gcg tcc acg cag acc tac gac agc aag agg aac gtt ggc gcc gtc      1344
Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val
          435          440          445

tcc tgg gac agc atc gac cag ctc ccg cca gag acc act gac gag cca      1392
Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro
          450          455          460

ctt gag aag gct tat agc cac cag ctg aac tac gtc atg tgc ttc ctc      1440
Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu
          465          470          475          480

atg caa ggc tct cgc ggc acc att ccg gtg ttc acc tgg acg cac aag      1488
Met Gln Gly Ser Arg Gly Thr Ile Pro Val Phe Thr Trp Thr His Lys
          485          490          495

agc gtt gac ttc ttc aac acc atc gac agc aag aag atc acc cag ctc      1536
Ser Val Asp Phe Phe Asn Thr Ile Asp Ser Lys Lys Ile Thr Gln Leu
          500          505          510

ccg ctt gtg aag gcc tac aag ctc cag agc ggc gcg agc gtg gtc gcc      1584
Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala
          515          520          525

ggg cct ggt ttc aca ggc ggc gac atc att cag tgt acg gag aac ggc      1632
Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly
          530          535          540

agc gcg gcc acc atc tac gtc acg ccg gac gtg agc tac tcc cag aag      1680
Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys
          545          550          555          560

tac aga gcc cgc atc cac tac gcc tcc acg agc cag atc acc ttc acc      1728
Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr
          565          570          575

ctg agc ctc gac ggc gcg ccc ctc aac cag tac tac ttc gac aag acc      1776
Leu Ser Leu Asp Gly Ala Pro Leu Asn Gln Tyr Tyr Phe Asp Lys Thr
          580          585          590

gtg aac aag ggc gac aca ctg acc tac aac agc ttc aac ctg gct agc      1824
Val Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser
          595          600          605

ttc tct acc cca ttc gag ctg agc ggc aac aac ctg cag atc ggc gtc      1872
Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val
          610          615          620

aca ggc ctc agc gcc ggc gac aag gtg tac atc gac aag att gag ttc      1920
Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe
          625          630          635          640

atc ccg gtc aac tga      1935
Ile Pro Val Asn

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<210> SEQ ID NO 11
<211> LENGTH: 644
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IP3-H5

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<400> SEQUENCE: 11

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Met His Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu
 1              5              10              15

Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr
20              25              30

Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met
35              40              45

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Thr	Ala	Asp	Asn	Asn	Thr	Glu	Ala	Leu	Asp	Ser	Ser	Thr	Thr	Arg	Asp
50						55					60				
Val	Ile	Gln	Lys	Gly	Ile	Ser	Val	Val	Gly	Asp	Leu	Leu	Gly	Val	Val
65					70					75					80
Gly	Phe	Pro	Phe	Gly	Gly	Ala	Leu	Val	Ser	Phe	Tyr	Thr	Asn	Phe	Leu
				85					90					95	
Asp	Thr	Ile	Trp	Pro	Ser	Glu	Asp	Pro	Leu	Lys	Ala	Phe	Met	Glu	Gln
			100					105					110		
Val	Glu	Ala	Leu	Ile	Asp	His	Lys	Ile	Ala	Asp	Tyr	Ala	Lys	Asn	Lys
		115					120					125			
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val
	130					135					140				
Ser	Ala	Leu	Ser	Ser	Trp	Gln	Glu	Asn	Pro	Val	Ser	Ser	Glu	Asn	Pro
145					150					155					160
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His
				165					170					175	
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Gly	Tyr	Glu	Val	Leu
			180					185					190		
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu
		195					200					205			
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp
	210					215					220				
Ile	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr
225					230						235				240
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly
				245					250					255	
Ser	Thr	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met
			260					265					270		
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val
		275					280					285			
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu
	290					295					300				
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr
305					310						315				320
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr
				325					330					335	
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Tyr	Gly
			340					345					350		
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro
			355					360				365			
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys
	370					375					380				
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr
385					390					395					400
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr
				405					410					415	
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp
			420					425					430		
Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Val	Gly	Ala	Val
			435				440					445			
Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro
	450					455					460				
Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu



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465	470	475	480
Met Gln Gly Ser Arg Gly Thr Ile Pro Val Phe Thr Trp Thr His Lys	485	490	495
Ser Val Asp Phe Phe Asn Thr Ile Asp Ser Lys Lys Ile Thr Gln Leu	500	505	510
Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala	515	520	525
Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly	530	535	540
Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys	545	550	555
Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr	565	570	575
Leu Ser Leu Asp Gly Ala Pro Leu Asn Gln Tyr Tyr Phe Asp Lys Thr	580	585	590
Val Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser	595	600	605
Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val	610	615	620
Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe	625	630	635
Ile Pro Val Asn			

&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 1935

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: IP3-H6

&lt;400&gt; SEQUENCE: 12

atgcacccta acaacaggtc agagcacgac acgatcaaga caaccgagaa caacgagggtg	60
ccgacgaacc acgtccagta cccactcgcc gaaacaccga accctaccct ggaggacctc	120
aactacaagg agttcctgag gatgacagcg gacaacaaca ccgaagccct cgactctagc	180
acgaccaggg acgtgattca gaagggcacc tctgtggctg gcgacctgct tggcggtgtc	240
gggttcccg tccggggcgc tctcgtcagc ttctacacga acttcttgga caccatctgg	300
ccgtccgaag accccctcaa ggcgttcatt gagcagggtg aggcctcat cgaccataag	360
atcgctgact atgccaagaa caaggctctg gcggagctgc aaggcctgca gaacaacttc	420
gaggactatg tgagcgccct cagctcttgg caggagaacc cagtcagctc cgagaaccgc	480
cacagccagg gccgcatcag ggagctgttc agccaggccg agagccactt ccgcaactcc	540
atgccgagct tcgccgtgag cggctacgag gtctctctcc tgactaccta tgcccaggct	600
gccaacaccc acttgcttct cctgaaggac gccagatct acggcgaaga gtggggctac	660
agctctgagg acatcgccga gttctaccac aggcagctca agctgacgca ggagtacacc	720
gaccactgag tgaagtggta caacgtcggc cttgacaagc tgagagggtc tacatacag	780
agctgggtca acttcaaccg ctataggcgc gagatgacac ttaccgtcct cgacctgatc	840
gcgctcttcc ctctgtacga cgtcagactt tactcgaagg gtgtcaagac cgagttgacc	900
agggacgtgc ttaccgaacc aatcgtcggc gtcaacaacc tgcgaggcta cgggaccacc	960
ttcagcaaca tcgagaacta catcaggaag ccgcacctgt tcgactacct gcaccgcatt	1020
cagttccaca ccaggctgca gcccggtac tacggcaacg acagcttcaa ctactggagc	1080

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ggcaactacg tgtctaccag acccagcadc ggctccaacg acatcatcac gtccccgttc 1140
tacggcaaca agagcagcga gccgggccag aacctggagt tcaacggcga gaaggcttat 1200
cgcgctgtcg ccaacaccaa cctcgcggtc tggccgagcg cgggtgtacag cggcgctcact 1260
aaggctcagtg tcagccagta caacgaccag accgacgagg cgtccacgca gacctacgac 1320
agcaagagga acgttgggcg cgtctcctgg gacagcatcg accagctccc gccagagacc 1380
actgacgagc cacttgagaa ggcttatagc caccagctga actacgtcat gtgcttcttc 1440
atgcaaggct ctcggggcac cattccgtg ttcacctgga cgcacaagag cgttgacttc 1500
ttcaacacca tcgacagcaa gaagatcacc cagctcccgc ttgtgaaggc ctacaagctc 1560
cagagcggcg cgagcgtggt cgccgggctt ggtttcacag gcggcgacat cattcagtg 1620
acggagaaacg gcagcgcggc caccatctac gtcacgcccg acgtgagcta ctcccagaag 1680
tacagagccc gcattcacta cgctccacg agccagatca ccttcacct gagcctcgac 1740
ggcgcgcccc tcaaccagta ctacttcgac aagaccgtga acaagggcga cacactgacc 1800
tacaacagct tcaacctggc tagcttctct accccattcg agctgagcgg caacaacctg 1860
cagatcggcg tcacaggcct cagcgccggc gacaagggtg acatcgacaa gattgagttc 1920
atccccgtca actga 1935

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<210> SEQ ID NO 13
<211> LENGTH: 1935
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IP3-H8
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1) ... (1935)

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<400> SEQUENCE: 13

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atg cac cct aac aac agg tca gag cac gac acg acc aag aca acc gag 48
Met His Pro Asn Asn Arg Ser Glu His Asp Thr Thr Lys Thr Thr Glu
1 5 10 15

aac aac gag gtg ccg acg aac cac gtc cag tac cca ctc gcc gaa aca 96
Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr
20 25 30

ccg aac cct acc ctg gag gac ctc aac tac aag gag ttc ctg agg atg 144
Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met
35 40 45

aca gcg gac aac aac acc gaa gcc ctc gac tct agc acg acc gag gac 192
Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Glu Asp
50 55 60

gtg att cag aag ggc atc tct gtg gtc ggc gac ctg ctt ggc gtt gtc 240
Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val
65 70 75 80

ggg ttc ccg ttc ggc ggc gct ctc gtc agc ttc tac acg aac ttc ttg 288
Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu
85 90 95

aac acc atc tgg ccg tcc gaa gac ccc ctc aag gcg ttc atg gag cag 336
Asn Thr Ile Trp Pro Ser Glu Asp Pro Leu Lys Ala Phe Met Glu Gln
100 105 110

gtg gag gcc ctc atc gac cag aag atc gct gac tat gcc aag aac aag 384
Val Glu Ala Leu Ile Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys
115 120 125

gct ctg gcg gag ctg caa ggc ctg cag aac aac ttc gag gac tat gtg 432
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val
130 135 140

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agc gcc ctc agc tct tgg cag gag aac cca gtc agc tcc gag aac ccg	480
Ser Ala Leu Ser Ser Trp Gln Glu Asn Pro Val Ser Ser Glu Asn Pro	
145 150 155 160	
cac agc cag ggc cgc atc agg gag ctg ttc agc cag gcc gag agc cac	528
His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His	
165 170 175	
ttc cgc aac tcc atg ccg agc ttc gcc gtg agc ggc tac gag gtc ctc	576
Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Gly Tyr Glu Val Leu	
180 185 190	
ttc ctg act acc tat gcc cag gct gcc aac acc cac ttg ctt ctc ctg	624
Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu	
195 200 205	
aag gac gcc cag atc tac ggc gaa gag tgg ggc tac gag aag gag gac	672
Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp	
210 215 220	
atc gcc gag ttc tac cac agg cat ctc aag ctg acg cag gag tac acc	720
Ile Ala Glu Phe Tyr His Arg His Leu Lys Leu Thr Gln Glu Tyr Thr	
225 230 235 240	
gac cac tgc gtg aag tgg tac aac gtc ggc ctt gac aag ctg aga ggg	768
Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly	
245 250 255	
tct aca tac gag agc tgg gtc aac ttc aac cgc tat agg cgc gag atg	816
Ser Thr Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met	
260 265 270	
aca ctt acc gtc ctc gac ctg atc gcg ctc ttc cct ctg tac gac gtc	864
Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val	
275 280 285	
aga ctt tac tcg aag ggt gtc aag acc gag ttg acc agg gac gtg ctt	912
Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Val Leu	
290 295 300	
acc gac cca atc gtc ggc gtc aac aac ctg cgc ggc tac ggg acc acc	960
Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr	
305 310 315 320	
ttc agc aac atc gag aac tac atc agg aag ccg cac ctg ttc gac tac	1008
Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr	
325 330 335	
ctg cac cgc att cag ttc cac acc agg ctg cag ccc ggc tac tac ggc	1056
Leu His Arg Ile Gln Phe His Thr Arg Leu Gln Pro Gly Tyr Tyr Gly	
340 345 350	
aac gac agc ttc aac tac tgg agc ggc aac tac gtg tct acc aga ccc	1104
Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro	
355 360 365	
agc atc ggc tcc aac gac atc atc acg tcc ccg ttc tac ggc aac aag	1152
Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys	
370 375 380	
agc agc gag ccg gtc cag aac ctg gag ttc aac ggc gag aag gtc tat	1200
Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr	
385 390 395 400	
cgc gct gtc gcc aac acc aac ctc gcg gtc tgg ccg agc gcg gtg tac	1248
Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr	
405 410 415	
agc ggc gtc act aag gtc gag ttc agc cag tac aac gac cag acc gac	1296
Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp	
420 425 430	
gag gcg tcc acg cag acc tac gac agc aag agg aac gtt ggc gcc gtc	1344
Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val	
435 440 445	
tcc tgg gac agc atc gac cag ctc ccg cca gag acc act gac gag cca	1392
Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro	

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450					455					460							
ctt	gag	aag	gct	tat	agc	cac	cag	ctg	aac	tac	gtc	atg	tgc	ttc	ctc	1440	
Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu		
465					470					475					480		
atg	caa	ggc	tct	cgc	ggc	acc	att	ccg	gtg	ttc	acc	tgg	aca	cac	gag	1488	
Met	Gln	Gly	Ser	Arg	Gly	Thr	Ile	Pro	Val	Phe	Thr	Trp	Thr	His	Glu		
					485					490					495		
agc	gtt	gac	ttc	ttc	aac	acc	atc	gac	agc	aag	aag	atc	acc	cag	ctc	1536	
Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ser	Lys	Lys	Ile	Thr	Gln	Leu		
					500					505					510		
ccg	ctt	gtg	aag	gcc	tac	aag	ctc	cag	agc	ggc	gcg	agc	gtg	gtc	gcc	1584	
Pro	Leu	Val	Lys	Ala	Tyr	Lys	Leu	Gln	Ser	Gly	Ala	Ser	Val	Val	Ala		
					515					520					525		
ggg	cct	ggt	ttc	aca	ggc	ggc	gac	atc	att	cag	tgt	acg	gag	aac	ggc	1632	
Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asp	Ile	Ile	Gln	Cys	Thr	Glu	Asn	Gly		
					530					535					540		
agc	gcg	gcc	acc	atc	tac	gtc	acg	ccg	gac	gtg	agc	cac	tcc	cag	aag	1680	
Ser	Ala	Ala	Thr	Ile	Tyr	Val	Thr	Pro	Asp	Val	Ser	His	Ser	Gln	Lys		
					545					550					555		560
tac	aga	gcc	cgc	atc	cac	tac	gcc	tcc	acg	agc	cag	atc	acc	ttc	acc	1728	
Tyr	Arg	Ala	Arg	Ile	His	Tyr	Ala	Ser	Thr	Ser	Gln	Ile	Thr	Phe	Thr		
					565					570					575		
ctg	agc	ctc	gac	ggc	gcg	ccc	ttc	aac	cag	tac	tac	ttc	gac	aag	acc	1776	
Leu	Ser	Leu	Asp	Gly	Ala	Pro	Phe	Asn	Gln	Tyr	Tyr	Phe	Asp	Lys	Thr		
					580					585					590		
atg	aac	aag	ggc	gac	acg	ctg	acc	tac	aac	agc	ttc	aac	ctg	gct	agc	1824	
Met	Asn	Lys	Gly	Asp	Thr	Leu	Thr	Tyr	Asn	Ser	Phe	Asn	Leu	Ala	Ser		
					595					600					605		
ttc	act	acc	cca	ttc	gag	ctg	agc	ggc	aac	aac	ctg	cag	atc	ggc	gtc	1872	
Phe	Thr	Thr	Pro	Phe	Glu	Leu	Ser	Gly	Asn	Asn	Leu	Gln	Ile	Gly	Val		
					610					615					620		
aca	ggc	ctc	agc	gcc	ggc	gac	aag	gtg	tac	atc	gac	aag	att	gag	ttc	1920	
Thr	Gly	Leu	Ser	Ala	Gly	Asp	Lys	Val	Tyr	Ile	Asp	Lys	Ile	Glu	Phe		
					625					630					635		640
atc	ccg	gtc	aac	tga												1935	
Ile	Pro	Val	Asn														

&lt;210&gt; SEQ ID NO 14

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: IP3-H8

&lt;400&gt; SEQUENCE: 14

Met His Pro Asn Asn Arg Ser Glu His Asp Thr Thr Lys Thr Thr Glu  
1 5 10 15

Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr  
20 25 30

Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met  
35 40 45

Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Glu Asp  
50 55 60

Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val  
65 70 75 80

Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu  
85 90 95

Asn Thr Ile Trp Pro Ser Glu Asp Pro Leu Lys Ala Phe Met Glu Gln  
100 105 110

Val	Glu	Ala	Leu	Ile	Asp	Gln	Lys	Ile	Ala	Asp	Tyr	Ala	Lys	Asn	Lys
115						120						125			
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val
130						135						140			
Ser	Ala	Leu	Ser	Ser	Trp	Gln	Glu	Asn	Pro	Val	Ser	Ser	Glu	Asn	Pro
145						150						155			
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His
			165						170						
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Gly	Tyr	Glu	Val	Leu
			180						185			190			
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu
195						200						205			
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp
210						215						220			
Ile	Ala	Glu	Phe	Tyr	His	Arg	His	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr
225						230						235			
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly
			245						250			255			
Ser	Thr	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met
260									265			270			
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val
275						280						285			
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu
290						295						300			
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr
305						310						315			
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr
			325						330			335			
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Tyr	Gly
340									345			350			
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro
355						360						365			
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys
370						375						380			
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr
385						390						395			
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr
			405						410			415			
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp
420									425			430			
Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Val	Gly	Ala	Val
435						440						445			
Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro
450						455						460			
Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu
465						470						475			
Met	Gln	Gly	Ser	Arg	Gly	Thr	Ile	Pro	Val	Phe	Thr	Trp	Thr	His	Glu
			485						490			495			
Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ser	Lys	Lys	Ile	Thr	Gln	Leu
500						505						510			
Pro	Leu	Val	Lys	Ala	Tyr	Lys	Leu	Gln	Ser	Gly	Ala	Ser	Val	Val	Ala
515						520						525			

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Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly  
530 535 540

Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser His Ser Gln Lys  
545 550 555 560

Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr  
565 570 575

Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr  
580 585 590

Met Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
595 600 605

Phe Thr Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
610 615 620

Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
625 630 635 640

Ile Pro Val Asn

<210> SEQ ID NO 15  
<211> LENGTH: 1935  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: IP3-H9

<400> SEQUENCE: 15

atgcacccta acaacaggtc agagcacgac acgaccaaga caaccgagaa caacgaggtg	60
ccgacgaacc acgtccagta cccactcgcc gaaacaccga accctaccct ggaggacctc	120
aactacaagg agttcctgag gatgacagcg gacaacaaca ccgaagccct cgactctagc	180
acgaccgagg acgtgattca gaagggcatc tctgtggtcg gcgacctgct tggcgttgtc	240
gggttccccg tcggcggcgc tctcgtcagc ttctacacga acttcttgaa caccatctgg	300
ccgtccgaag accccctcaa ggcggttcag gagcaggtgg agggccctcat cgaccagaag	360
atcgctgact atgccaagaa caaggctctg gcggagctgc aaggcctgca gaacaacttc	420
gaggactatg tgagcgccct cagctcttgg caggagaacc cagtcagctc cgagaacccg	480
cacagccagg gccgcatcag ggagctgttc agccaggccg agagccactt ccgcaactcc	540
atgccgagct tcgccgtgag cggctacgag gtcctcttcc tgactaccta tgcccaggct	600
gccaacaccc acttgcttct cctgaaggac gcccagatct acggcgaaga gtggggctac	660
agctctgagg acatcgccga gttctaccac aggcattcga agctgacgca ggagtacacc	720
gaccactgcg tgaagtggta caacgtcggc cttgacaagc tgagagggtc tacatacgag	780
agctgggtca acttcaaccg ctataggcgc gagatgacac ttaccgtcct cgacctgatc	840
gcgctcttcc ctctgtacga cgtcagactt tactcgaagg gtgtcaagac cgagttgacc	900
agggacgtgc ttaccgaccc aatcgtcggc gtcaacaacc tgcgcggcta cgggaccacc	960
ttcagcaaca tcgagaacta catcaggaag ccgcacctgt tcgactacct gcaccgcatt	1020
cagttccaca ccaggctgca gccggctac tacggcaacg acagcttcaa ctactggagc	1080
ggcaactacg tgtctaccag acccagcatc ggctccaacg acatcatcac gtcccgttc	1140
tacggcaaca agagcagcga gccggtccag aacctggagt tcaacggcga gaaggtctat	1200
cgcgctgtcg ccaacaccaa cctcgcggtc tggccgagcg cgggtgtacag cggcgtcact	1260
aaggtcgagt tcagccagta caacgaccag accgacgagg cgtccacgca gacctacgac	1320
agcaagagga acgttgggcg cgtctcctgg gacagcatcg accagctccc gccagagacc	1380

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actgacgagc cacttgagaa ggcttatagc caccagctga actacgtcat gtgcttcctc 1440
atgcaaggct ctgcgggcac cattccgggtg ttcacctgga cacacgagag cgttgacttc 1500
ttcaacacca tcgacagcaa gaagatcacc cagctccgcg ttgtgaaggc ctacaagctc 1560
cagagcggcg cgagcgtggt cgccgggctt ggtttcacag gcggcgacat cattcagtgt 1620
acggagaacg gcagcgcggc caccatctac gtcacgcggc acgtgagcca ctcccagaag 1680
tacagagccc gcattccacta cgctccacg agccagatca ccttcacct gagcctcgac 1740
ggcgcgcctt tcaaccagta ctacttcgac aagaccatga acaaggcgca cagctgacc 1800
tacaacagct tcaacctggc tagcttcact acccattcg agctgagcgg caacaacctg 1860
cagatcggcg tcacaggcct cagcgcggc gacaaggtgt acatcgacaa gattgagttc 1920
atcccgttca actga 1935

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&lt;210&gt; SEQ ID NO 16

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Bacillus thuringiensis*

&lt;400&gt; SEQUENCE: 16

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu
 1             5             10             15
Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr
      20             25             30
Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met
      35             40             45
Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp
      50             55             60
Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val
      65             70             75             80
Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu
      85             90             95
Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln
      100            105            110
Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys
      115            120            125
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val
      130            135            140
Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro
      145            150            155            160
His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His
      165            170            175
Phe Arg Asn Ser Met Pro Ser Phe Ala Ile Ser Gly Tyr Glu Val Leu
      180            185            190
Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Phe Leu Leu
      195            200            205
Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp
      210            215            220
Ile Ala Glu Phe Tyr Lys Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr
      225            230            235            240
Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly
      245            250            255
Ser Ser Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met
      260            265            270

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Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val
  275                      280                      285

Arg Leu Tyr Pro Lys Glu Val Lys Thr Glu Leu Thr Arg Asp Val Leu
  290                      295                      300

Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr
  305                      310                      315                      320

Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr
  325                      330                      335

Leu His Arg Ile Gln Phe His Thr Arg Phe Gln Pro Gly Tyr Tyr Gly
  340                      345                      350

Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro
  355                      360                      365

Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys
  370                      375                      380

Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr
  385                      390                      395                      400

Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr
  405                      410                      415

Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp
  420                      425                      430

Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val
  435                      440                      445

Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro
  450                      455                      460

Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu
  465                      470                      475                      480

Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys
  485                      490                      495

Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu
  500                      505                      510

Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala
  515                      520                      525

Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly
  530                      535                      540

Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys
  545                      550                      555                      560

Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr
  565                      570                      575

Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr
  580                      585                      590

Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser
  595                      600                      605

Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val
  610                      615                      620

Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe
  625                      630                      635                      640

Ile Pro Val Asn

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&lt;210&gt; SEQ ID NO 17

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Bacillus thuringiensis*

&lt;400&gt; SEQUENCE: 17



Met 1	Asn	Pro	Asn	Asn 5	Arg	Ser	Glu	His	Asp 10	Thr	Ile	Lys	Thr	Thr 15	Glu
Asn	Asn	Glu	Val 20	Pro	Thr	Asn	His	Val 25	Gln	Tyr	Pro	Leu	Ala 30	Glu	Thr
Pro	Asn	Pro	Thr	Leu	Glu	Asp 40	Leu	Asn	Tyr	Lys	Glu	Phe 45	Leu	Arg	Met
Thr	Ala 50	Asp	Asn	Asn	Thr	Glu 55	Ala	Leu	Asp	Ser	Ser 60	Thr	Thr	Lys	Asp
Val 65	Ile	Gln	Lys	Gly	Ile 70	Ser	Val	Val	Gly	Asp 75	Leu	Leu	Gly	Val	Val 80
Gly	Phe	Pro	Phe	Gly 85	Gly	Ala	Leu	Val	Ser 90	Phe	Tyr	Thr	Asn	Phe 95	Leu
Asn	Thr	Ile	Trp 100	Pro	Ser	Glu	Asp	Pro 105	Trp	Lys	Ala	Phe	Met 110	Glu	Gln
Val	Glu	Ala	Leu	Met	Asp	Gln	Lys 120	Ile	Ala	Asp	Tyr	Ala 125	Lys	Asn	Lys
Ala 130	Leu	Ala	Glu	Leu	Gln	Gly 135	Leu	Gln	Asn	Asn	Val 140	Glu	Asp	Tyr	Val
Ser 145	Ala	Leu	Ser	Ser	Trp 150	Gln	Lys	Asn	Pro	Val 155	Ser	Ser	Arg	Asn	Pro 160
His	Ser	Gln	Gly	Arg 165	Ile	Arg	Glu	Leu	Phe 170	Ser	Gln	Ala	Glu	Ser 175	His
Phe	Arg	Asn	Ser	Met 180	Pro	Ser	Phe	Ala 185	Ile	Ser	Gly	Tyr	Glu 190	Val	Leu
Phe	Leu	Thr 195	Thr	Tyr	Ala	Gln	Ala 200	Ala	Asn	Thr	His	Leu 205	Phe	Leu	Leu
Lys 210	Asp	Ala	Gln	Ile	Tyr	Gly 215	Glu	Glu	Trp	Gly 220	Tyr	Glu	Lys	Glu	Asp
Ile 225	Ala	Glu	Phe	Tyr	Lys 230	Arg	Gln	Leu	Lys	Leu 235	Thr	Gln	Glu	Tyr	Thr 240
Asp	His	Cys	Val	Lys 245	Trp	Tyr	Asn	Val	Gly 250	Leu	Asp	Lys	Leu	Arg 255	Gly
Ser	Ser	Tyr	Glu	Ser	Trp	Val	Asn	Phe 265	Asn	Arg	Tyr	Arg 270	Arg	Glu	Met
Thr	Leu	Thr 275	Val	Leu	Asp	Leu	Ile 280	Ala	Leu	Phe	Pro	Leu 285	Tyr	Asp	Val
Arg 290	Leu	Tyr	Pro	Lys	Glu	Val 295	Lys	Thr	Glu	Leu	Thr 300	Arg	Asp	Val	Leu
Thr 305	Asp	Pro	Ile	Val	Gly 310	Val	Asn	Asn	Leu	Arg 315	Gly	Tyr	Gly	Thr	Thr 320
Phe	Ser	Asn	Ile	Glu 325	Asn	Tyr	Ile	Arg	Lys 330	Pro	His	Leu	Phe	Asp 335	Tyr
Leu	His	Arg	Ile	Gln 340	Phe	His	Thr	Arg 345	Phe	Gln	Pro	Gly 350	Tyr	Tyr	Gly
Asn	Asp	Ser 355	Phe	Asn	Tyr	Trp	Ser 360	Gly	Asn	Tyr	Val	Ser 365	Thr	Arg	Pro
Ser 370	Ile	Gly	Ser	Asn	Asp 375	Ile	Ile	Thr	Ser	Pro	Phe 380	Tyr	Gly	Asn	Lys
Ser 385	Ser	Glu	Pro	Val	Gln 390	Asn	Leu	Glu	Phe	Asn 395	Gly	Glu	Lys	Val	Tyr 400
Arg	Ala	Val	Ala	Asn 405	Thr	Asn	Leu	Ala	Val 410	Trp	Pro	Ser	Ala	Val 415	Tyr
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asn

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420	425	430
Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val		
435	440	445
Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro		
450	455	460
Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu		
465	470	475
Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys		
485	490	495
Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu		
500	505	510
Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala		
515	520	525
Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly		
530	535	540
Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys		
545	550	555
Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr		
565	570	575
Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr		
580	585	590
Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser		
595	600	605
Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val		
610	615	620
Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe		
625	630	635
640		
Ile Pro Val Asn		

&lt;210&gt; SEQ ID NO 18

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 18

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu		
1	5	10
Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr		
20	25	30
Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met		
35	40	45
Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp		
50	55	60
Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val		
65	70	75
Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu		
85	90	95
Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln		
100	105	110
Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys		
115	120	125
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val		
130	135	140
Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro		

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145					150					155					160
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His
				165					170					175	
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Ile	Ser	Gly	Tyr	Glu	Val	Leu
		180						185					190		
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Phe	Leu	Leu
	195						200					205			
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp
210						215					220				
Ile	Ala	Glu	Phe	Tyr	Lys	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr
225					230					235					240
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly
			245						250					255	
Ser	Ser	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met
		260						265						270	
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val
	275						280					285			
Arg	Leu	Tyr	Pro	Lys	Glu	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu
290						295					300				
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr
305					310					315					320
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr
			325						330					335	
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Phe	Gln	Pro	Gly	Tyr	Tyr	Gly
		340						345					350		
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro
	355						360					365			
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys
370					375						380				
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr
385					390					395					400
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr
			405						410					415	
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp
		420						425					430		
Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Val	Gly	Ala	Val
	435						440					445			
Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro
450					455						460				
Leu	Glu	Lys	Gly	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu
465					470					475					480
Met	Gln	Gly	Ser	Arg	Gly	Thr	Ile	Pro	Val	Leu	Thr	Trp	Thr	His	Lys
			485						490					495	
Ser	Val	Asp	Phe	Phe	Asn	Met	Ile	Asp	Ser	Lys	Lys	Ile	Thr	Gln	Leu
		500						505						510	
Pro	Leu	Val	Lys	Ala	Tyr	Lys	Leu	Gln	Ser	Gly	Ala	Ser	Val	Val	Ala
	515						520					525			
Gly	Pro	Arg	Phe	Thr	Gly	Gly	Asp	Ile	Ile	Gln	Cys	Thr	Glu	Asn	Gly
530						535					540				
Ser	Ala	Ala	Thr	Ile	Tyr	Val	Thr	Pro	Asp	Val	Ser	Tyr	Ser	Gln	Lys
545					550					555					560
Tyr	Arg	Ala	Arg	Ile	His	Tyr	Ala	Ser	Thr	Ser	Gln	Ile	Thr	Phe	Thr
				565					570					575	

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Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr  
 580 585 590  
 Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
 595 600 605  
 Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
 610 615 620  
 Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
 625 630 635 640  
 Ile Pro Val Asn  
  
 <210> SEQ ID NO 19  
 <211> LENGTH: 644  
 <212> TYPE: PRT  
 <213> ORGANISM: *Bacillus thuringiensis*  
  
 <400> SEQUENCE: 19  
 Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu  
 1 5 10 15  
 Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr  
 20 25 30  
 Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met  
 35 40 45  
 Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp  
 50 55 60  
 Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val  
 65 70 75 80  
 Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu  
 85 90 95  
 Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln  
 100 105 110  
 Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys  
 115 120 125  
 Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val  
 130 135 140  
 Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro  
 145 150 155 160  
 His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His  
 165 170 175  
 Phe Arg Asn Ser Met Pro Ser Phe Ala Ile Ser Gly Tyr Glu Val Leu  
 180 185 190  
 Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Phe Leu Leu  
 195 200 205  
 Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp  
 210 215 220  
 Ile Ala Glu Phe Tyr Lys Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr  
 225 230 235 240  
 Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly  
 245 250 255  
 Ser Ser Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met  
 260 265 270  
 Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val  
 275 280 285  
 Arg Leu Tyr Pro Lys Glu Val Lys Thr Glu Leu Thr Arg Asp Val Leu  
 290 295 300

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Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr  
 305 310 315 320  
 Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr  
 325 330 335  
 Leu His Arg Ile Gln Phe His Thr Arg Phe Gln Pro Gly Tyr Tyr Gly  
 340 345 350  
 Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro  
 355 360 365  
 Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys  
 370 375 380  
 Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr  
 385 390 395 400  
 Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr  
 405 410 415  
 Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp  
 420 425 430  
 Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val  
 435 440 445  
 Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro  
 450 455 460  
 Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu  
 465 470 475 480  
 Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys  
 485 490 495  
 Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu  
 500 505 510  
 Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala  
 515 520 525  
 Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Ala  
 530 535 540  
 Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys  
 545 550 555 560  
 Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr  
 565 570 575  
 Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr  
 580 585 590  
 Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
 595 600 605  
 Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
 610 615 620  
 Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
 625 630 635 640  
 Ile Pro Val Asn

&lt;210&gt; SEQ ID NO 20

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 20

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu  
 1 5 10 15  
 Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr  
 20 25 30

Pro 35	Asn 50	Pro 65	Thr 80	Leu 85	Glu 90	Asp 100	Leu 110	Asn 120	Tyr 130	Lys 140	Glu 150	Phe 160	Leu 170	Arg 180	Met 190
Thr 50	Ala 65	Asp 80	Asn 85	Asn 90	Thr 100	Glu 110	Ala 120	Leu 130	Asp 140	Ser 150	Ser 160	Thr 170	Thr 180	Lys 190	Asp 200
Val 65	Ile 80	Gln 85	Lys 90	Gly 95	Ile 100	Ser 110	Val 120	Val 130	Gly 140	Asp 150	Leu 160	Leu 170	Gly 180	Val 190	Val 200
Gly 80	Phe 90	Pro 100	Phe 110	Gly 120	Gly 130	Ala 140	Leu 150	Val 160	Ser 170	Phe 180	Tyr 190	Thr 200	Asn 210	Phe 220	Leu 230
Asn 100	Thr 110	Ile 120	Trp 130	Pro 140	Ser 150	Glu 160	Asp 170	Pro 180	Trp 190	Lys 200	Ala 210	Phe 220	Met 230	Glu 240	Gln 250
Val 115	Glu 125	Ala 135	Leu 145	Met 155	Asp 165	Gln 175	Lys 185	Ile 195	Ala 205	Asp 215	Tyr 225	Ala 235	Lys 245	Asn 255	Lys 265
Ala 130	Leu 140	Ala 150	Glu 160	Leu 170	Gln 180	Gly 190	Leu 200	Gln 210	Asn 220	Asn 230	Val 240	Glu 250	Asp 260	Tyr 270	Val 280
Ser 145	Ala 155	Leu 165	Ser 175	Ser 185	Trp 195	Gln 205	Lys 215	Asn 225	Pro 235	Val 245	Ser 255	Ser 265	Arg 275	Asn 285	Pro 295
His 160	Ser 170	Gln 180	Gly 190	Arg 200	Ile 210	Arg 220	Glu 230	Leu 240	Phe 250	Ser 260	Gln 270	Ala 280	Glu 290	Ser 300	His 310
Phe 180	Arg 190	Asn 200	Ser 210	Met 220	Pro 230	Ser 240	Phe 250	Ala 260	Ile 270	Ser 280	Gly 290	Tyr 300	Glu 310	Val 320	Leu 330
Phe 195	Leu 205	Thr 215	Thr 225	Tyr 235	Ala 245	Gln 255	Ala 265	Ala 275	Asn 285	Thr 295	His 305	Leu 315	Phe 325	Leu 335	Leu 345
Lys 210	Asp 220	Ala 230	Gln 240	Ile 250	Tyr 260	Gly 270	Glu 280	Glu 290	Trp 300	Gly 310	Tyr 320	Glu 330	Lys 340	Glu 350	Asp 360
Ile 225	Ala 235	Glu 245	Phe 255	Tyr 265	Lys 275	Arg 285	Gln 295	Leu 305	Lys 315	Leu 325	Thr 335	Gln 345	Glu 355	Tyr 365	Thr 375
Asp 240	His 250	Cys 260	Val 270	Lys 280	Trp 290	Tyr 300	Asn 310	Val 320	Gly 330	Leu 340	Asp 350	Lys 360	Leu 370	Arg 380	Gly 390
Ser 260	Ser 270	Tyr 280	Glu 290	Ser 300	Trp 310	Val 320	Asn 330	Phe 340	Asn 350	Arg 360	Tyr 370	Arg 380	Arg 390	Glu 400	Met 410
Thr 275	Leu 285	Thr 295	Val 305	Leu 315	Asp 325	Leu 335	Ile 345	Ala 355	Leu 365	Phe 375	Pro 385	Leu 395	Tyr 405	Asp 415	Val 425
Arg 290	Leu 300	Tyr 310	Pro 320	Lys 330	Glu 340	Val 350	Lys 360	Thr 370	Glu 380	Leu 390	Thr 400	Arg 410	Asp 420	Val 430	Leu 440
Thr 305	Asp 315	Pro 325	Ile 335	Val 345	Gly 355	Val 365	Asn 375	Asn 385	Leu 395	Arg 405	Gly 415	Tyr 425	Gly 435	Thr 445	Thr 455
Phe 320	Ser 330	Asn 340	Ile 350	Glu 360	Asn 370	Tyr 380	Ile 390	Arg 400	Lys 410	Pro 420	His 430	Leu 440	Phe 450	Asp 460	Tyr 470
Leu 335	His 345	Arg 355	Ile 365	Gln 375	Phe 385	His 395	Thr 405	Arg 415	Phe 425	Gln 435	Pro 445	Gly 455	Tyr 465	Tyr 475	Gly 485
Asn 355	Asp 365	Ser 375	Phe 385	Asn 395	Tyr 405	Trp 415	Ser 425	Gly 435	Asn 445	Tyr 455	Val 465	Ser 475	Thr 485	Arg 495	Pro 505
Ser 370	Ile 380	Gly 390	Ser 400	Asn 410	Asp 420	Ile 430	Ile 440	Thr 450	Ser 460	Pro 470	Phe 480	Tyr 490	Gly 500	Asn 510	Lys 520
Ser 385	Ser 395	Glu 405	Pro 415	Val 425	Gln 435	Asn 445	Leu 455	Glu 465	Phe 475	Asn 485	Gly 495	Glu 505	Lys 515	Val 525	Tyr 535
Arg 400	Ala 410	Val 420	Ala 430	Asn 440	Thr 450	Asn 460	Leu 470	Ala 480	Val 490	Trp 500	Pro 510	Ser 520	Ala 530	Val 540	Tyr 550
Ser 420	Gly 430	Val 440	Thr 450	Lys 460	Val 470										

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Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro  
 450 455 460  
 Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu  
 465 470 475 480  
 Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys  
 485 490 495  
 Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu  
 500 505 510  
 Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala  
 515 520 525  
 Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly  
 530 535 540  
 Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys  
 545 550 555 560  
 Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr  
 565 570 575  
 Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr  
 580 585 590  
 Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
 595 600 605  
 Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
 610 615 620  
 Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
 625 630 635 640  
 Ile Pro Val Asn

<210> SEQ ID NO 21  
 <211> LENGTH: 644  
 <212> TYPE: PRT  
 <213> ORGANISM: Bacillus thuringiensis

<400> SEQUENCE: 21

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu  
 1 5 10 15  
 Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr  
 20 25 30  
 Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met  
 35 40 45  
 Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp  
 50 55 60  
 Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val  
 65 70 75 80  
 Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu  
 85 90 95  
 Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln  
 100 105 110  
 Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys  
 115 120 125  
 Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val  
 130 135 140  
 Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro  
 145 150 155 160  
 His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His  
 165 170 175

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Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Ile	Ser	Gly	Tyr	Glu	Val	Leu
		180						185					190		
Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Phe	Leu	Leu
		195					200					205			
Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu	Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp
	210					215					220				
Ile	Ala	Glu	Phe	Tyr	Lys	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr
225					230					235					240
Asp	His	Cys	Val	Lys	Trp	Tyr	Asn	Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly
			245						250					255	
Ser	Ser	Tyr	Glu	Ser	Trp	Val	Asn	Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met
		260						265					270		
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val
		275					280					285			
Arg	Leu	Tyr	Pro	Lys	Glu	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu
	290					295					300				
Thr	Asp	Pro	Ile	Val	Gly	Val	Asn	Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr
305					310					315					320
Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr
			325						330					335	
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Phe	Gln	Pro	Gly	Tyr	Tyr	Gly
			340					345					350		
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro
		355					360					365			
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys
	370					375					380				
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr
385					390					395					400
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr
			405						410					415	
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp
			420					425					430		
Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Val	Gly	Ala	Val
		435					440					445			
Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro
	450					455					460				
Leu	Glu	Lys	Gly	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu
465					470					475					480
Met	Gln	Gly	Ser	Arg	Gly	Thr	Ile	Pro	Val	Leu	Thr	Trp	Thr	His	Lys
			485						490					495	
Ser	Val	Asp	Phe	Phe	Asn	Met	Ile	Asp	Ser	Lys	Lys	Ile	Thr	Gln	Leu
		500						505					510		
Pro	Leu	Val	Lys	Ala	Tyr	Lys	Leu	Gln	Ser	Gly	Ala	Ser	Val	Val	Ala
		515					520					525			
Gly	Pro	Arg	Phe	Thr	Gly	Gly	Asp	Ile	Ile	Gln	Cys	Thr	Glu	Asn	Gly
	530					535					540				
Ser	Ala	Ala	Thr	Ile	Tyr	Val	Thr	Pro	Asp	Val	Ser	Tyr	Ser	Gln	Lys
545					550					555					560
Tyr	Arg	Ala	Arg	Ile	His	Tyr	Ala	Ser	Thr	Ser	Gln	Ile	Thr	Phe	Thr
			565					570						575	
Leu	Ser	Leu	Asp	Gly	Ala	Pro	Phe	Asn	Gln	Tyr	Tyr	Phe	Asp	Lys	Thr
		580						585					590		
Ile	Asn	Lys	Gly	Asp	Thr	Leu	Thr	Tyr	Asn	Ser	Phe	Asn	Leu	Ala	Ser



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595	600	605
Phe Ser Thr Pro Phe Glu	Leu Ser Gly Asn Asn	Leu Gln Ile Gly Val
610	615	620
Thr Gly Leu Ser Ala Gly	Asp Lys Val Tyr Ile	Asp Lys Ile Glu Phe
625	630	635 640
Ile Pro Val Asn		
<210> SEQ ID NO 22		
<211> LENGTH: 644		
<212> TYPE: PRT		
<213> ORGANISM: Bacillus thuringiensis		
<400> SEQUENCE: 22		
Met Asn Pro Asn Asn Arg Ser	Glu His Asp Thr Ile Lys Thr Thr Glu	
1	5 10 15	
Asn Asn Glu Val Pro Thr	Asn His Val Gln Tyr Pro Leu Ala Glu Thr	
20	25 30	
Pro Asn Pro Thr Leu Glu Asp	Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35	40 45	
Thr Ala Asp Asn Asn Thr	Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp	
50	55 60	
Val Ile Gln Lys Gly Ile Ser	Val Val Gly Asp Leu Leu Gly Val Val	
65	70 75 80	
Gly Phe Pro Phe Gly Gly Ala	Leu Val Ser Phe Tyr Thr Asn Phe Leu	
85	90 95	
Asn Thr Ile Trp Pro Ser Glu	Asp Pro Trp Lys Ala Phe Met Glu Gln	
100	105 110	
Val Glu Ala Leu Met Asp Gln	Lys Ile Ala Asp Tyr Ala Lys Asn Lys	
115	120 125	
Ala Leu Ala Glu Leu Gln Gly	Leu Gln Asn Asn Val Glu Asp Tyr Val	
130	135 140	
Ser Ala Leu Ser Ser Trp Gln	Lys Asn Pro Val Ser Ser Arg Asn Pro	
145	150 155 160	
His Ser Gln Gly Arg Ile Arg	Glu Leu Phe Ser Gln Ala Glu Ser His	
165	170 175	
Phe Arg Asn Ser Met Pro Ser	Phe Ala Ile Ser Gly Tyr Glu Val Leu	
180	185 190	
Phe Leu Thr Thr Tyr Ala Gln	Ala Ala Asn Thr His Leu Phe Leu Leu	
195	200 205	
Lys Asp Ala Gln Ile Tyr Gly	Glu Glu Trp Gly Tyr Glu Lys Glu Asp	
210	215 220	
Ile Ala Glu Phe Tyr Lys Arg	Gln Leu Lys Leu Thr Gln Glu Tyr Thr	
225	230 235 240	
Asp His Cys Val Lys Trp Tyr	Asn Val Gly Leu Asp Lys Leu Arg Gly	
245	250 255	
Ser Ser Tyr Glu Ser Trp Val	Asn Phe Asn Arg Tyr Arg Arg Glu Met	
260	265 270	
Thr Leu Thr Val Leu Asp Leu	Ile Ala Leu Phe Pro Leu Tyr Asp Val	
275	280 285	
Arg Leu Tyr Pro Lys Glu Val	Lys Thr Glu Leu Thr Arg Asp Val Leu	
290	295 300	
Thr Asp Pro Ile Val Gly Val	Asn Asn Leu Arg Gly Tyr Gly Thr Thr	
305	310 315 320	
Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr		

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325					330					335					
Leu	His	Arg	Ile	Gln	Phe	His	Thr	Arg	Phe	Gln	Pro	Gly	Tyr	Tyr	Gly
			340					345					350		
Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro
		355					360					365			
Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys
	370					375					380				
Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu	Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr
	385					390					395				400
Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu	Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr
				405					410					415	
Ser	Gly	Val	Thr	Lys	Val	Glu	Phe	Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp
			420					425					430		
Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Val	Gly	Ala	Val
		435					440					445			
Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro
	450					455					460				
Leu	Glu	Lys	Gly	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Val	Met	Cys	Phe	Leu
	465					470					475				480
Met	Gln	Gly	Ser	Arg	Gly	Thr	Ile	Pro	Val	Leu	Thr	Trp	Thr	His	Lys
				485					490					495	
Ser	Val	Asp	Phe	Phe	Asn	Met	Ile	Asp	Ser	Lys	Lys	Ile	Thr	Gln	Leu
			500					505					510		
Pro	Leu	Val	Lys	Ala	Tyr	Lys	Leu	Gln	Ser	Gly	Ala	Ser	Val	Val	Ala
		515					520					525			
Gly	Pro	Arg	Phe	Thr	Gly	Gly	Asp	Ile	Ile	Gln	Cys	Thr	Glu	Asn	Gly
	530					535					540				
Ser	Ala	Ala	Thr	Ile	Tyr	Val	Thr	Pro	Asp	Val	Ser	Tyr	Ser	Gln	Lys
	545					550					555				560
Tyr	Arg	Ala	Arg	Ile	His	Tyr	Ala	Ser	Thr	Ser	Gln	Ile	Thr	Phe	Thr
				565					570					575	
Leu	Ser	Leu	Asp	Gly	Ala	Pro	Phe	Asn	Gln	Tyr	Tyr	Phe	Asp	Lys	Thr
			580					585					590		
Ile	Asn	Lys	Gly	Asp	Thr	Leu	Thr	Tyr	Asn	Ser	Phe	Asn	Leu	Ala	Ser
		595					600					605			
Phe	Ser	Thr	Pro	Phe	Glu	Leu	Ser	Gly	Asn	Asn	Leu	Gln	Ile	Gly	Val
	610					615					620				
Thr	Gly	Leu	Ser	Ala	Gly	Asp	Lys	Val	Tyr	Ile	Asp	Lys	Ile	Glu	Phe
	625					630					635			640	
Ile	Pro	Val	Asn												

&lt;210&gt; SEQ ID NO 23

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 23

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Thr	Thr	Glu
1				5					10					15	

Asn	Asn	Glu	Val	Pro	Thr	Asn	His	Val	Gln	Tyr	Pro	Leu	Ala	Glu	Thr
		20					25					30			

Pro	Asn	Pro	Thr	Leu	Glu	Asp	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
	35						40					45			

Thr	Ala	Asp	Asn	Asn	Thr	Glu	Ala	Leu	Asp	Ser	Ser	Thr	Thr	Lys	Asp
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50	55	60
Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val		
65	70	75 80
Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu		
	85	90 95
Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln		
	100	105 110
Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys		
	115	120 125
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val		
	130	135 140
Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro		
145	150	155 160
His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His		
	165	170 175
Phe Arg Asn Ser Met Pro Ser Phe Ala Ile Ser Gly Tyr Glu Val Leu		
	180	185 190
Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Phe Leu Leu		
	195	200 205
Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp		
	210	215 220
Ile Ala Glu Phe Tyr Lys Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr		
225	230	235 240
Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly		
	245	250 255
Ser Ser Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met		
	260	265 270
Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val		
	275	280 285
Arg Leu Tyr Pro Lys Glu Val Lys Thr Glu Leu Thr Arg Asp Val Leu		
	290	295 300
Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr		
305	310	315 320
Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr		
	325	330 335
Leu His Arg Ile Gln Phe His Thr Gln Phe Gln Pro Gly Tyr Tyr Gly		
	340	345 350
Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro		
	355	360 365
Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys		
	370	375 380
Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr		
385	390	395 400
Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr		
	405	410 415
Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp		
	420	425 430
Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val		
	435	440 445
Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro		
	450	455 460
Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu		
465	470	475 480

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Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys  
485 490 495

Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu  
500 505 510

Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala  
515 520 525

Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly  
530 535 540

Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys  
545 550 555 560

Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr  
565 570 575

Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr  
580 585 590

Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
595 600 605

Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
610 615 620

Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
625 630 635 640

Ile Pro Val Asn

&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 644

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 24

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu  
1 5 10 15

Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr  
20 25 30

Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met  
35 40 45

Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp  
50 55 60

Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val  
65 70 75 80

Gly Phe Pro Phe Gly Glu Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu  
85 90 95

Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln  
100 105 110

Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys  
115 120 125

Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val  
130 135 140

Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro  
145 150 155 160

His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His  
165 170 175

Phe Arg Asn Ser Met Pro Ser Phe Ala Ile Ser Gly Tyr Glu Val Leu  
180 185 190

Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Phe Leu Leu  
195 200 205

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Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Lys Lys Glu Asp  
 210 215 220  
 Ile Ala Glu Phe Leu Lys Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr  
 225 230 235 240  
 Asp His Phe Val Gln Trp Tyr Tyr Val Gly Leu Asp Lys Ile Arg Gly  
 245 250 255  
 Ser Phe Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met  
 260 265 270  
 Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val  
 275 280 285  
 Arg Leu Tyr Pro Lys Glu Val Lys Thr Glu Leu Thr Arg Asp Val Leu  
 290 295 300  
 Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr  
 305 310 315 320  
 Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr  
 325 330 335  
 Leu His Arg Ile Gln Phe His Thr Arg Phe Gln Pro Gly Tyr Tyr Gly  
 340 345 350  
 Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro  
 355 360 365  
 Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys  
 370 375 380  
 Ser Ser Glu Pro Val Gln Asn Leu Gly Phe Asn Gly Glu Lys Val Tyr  
 385 390 395 400  
 Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Asn  
 405 410 415  
 Ser Gly Val Thr Lys Val Lys Phe Ser Gln Tyr Asn Asp Gln Thr Asp  
 420 425 430  
 Glu Ala Ser Thr Gln Thr Ser Asp Ser Lys Arg Asn Val Gly Ala Val  
 435 440 445  
 Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Ala Thr Asp Glu Pro  
 450 455 460  
 Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu  
 465 470 475 480  
 Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys  
 485 490 495  
 Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu  
 500 505 510  
 Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala  
 515 520 525  
 Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly  
 530 535 540  
 Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys  
 545 550 555 560  
 Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr  
 565 570 575  
 Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr  
 580 585 590  
 Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser  
 595 600 605  
 Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val  
 610 615 620

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Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe  
625 630 635 640

Ile Pro Val Asn

<210> SEQ ID NO 25

<211> LENGTH: 644

<212> TYPE: PRT

<213> ORGANISM: *Bacillus thuringiensis*

<400> SEQUENCE: 25

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu  
1 5 10 15

Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr  
20 25 30

Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met  
35 40 45

Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp  
50 55 60

Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val  
65 70 75 80

Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu  
85 90 95

Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln  
100 105 110

Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys  
115 120 125

Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val  
130 135 140

Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro  
145 150 155 160

His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His  
165 170 175

Phe Arg Asn Ser Met Pro Ser Phe Ala Ile Ser Gly Tyr Glu Val Leu  
180 185 190

Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Ile His Leu Phe Leu Leu  
195 200 205

Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp  
210 215 220

Ile Ala Glu Phe Tyr Lys Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr  
225 230 235 240

Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly  
245 250 255

Ser Ser Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met  
260 265 270

Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val  
275 280 285

Arg Leu Tyr Pro Lys Glu Val Lys Thr Glu Leu Thr Arg Asp Val Leu  
290 295 300

Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr  
305 310 315 320

Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr  
325 330 335

Leu His Arg Ile Gln Phe His Thr Arg Phe Arg Pro Gly Tyr Tyr Gly  
340 345 350

[illegible]

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<210> SEQ ID NO 26
<211> LENGTH: 644
<212> TYPE: PRT
<213> ORGANISM: Bacillus thuringiensis
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<400> SEQUENCE: 26

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Thr	Thr	Glu
1				5					10					15	
Asn	Asn	Glu	Val	Pro	Thr	Asn	His	Val	Gln	Tyr	Pro	Leu	Ala	Glu	Thr
			20					25					30		
Pro	Asn	Pro	Thr	Leu	Glu	Asp	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
		35					40					45			
Thr	Ala	Asp	Asn	Asn	Thr	Glu	Ala	Leu	Asp	Ser	Ser	Thr	Thr	Lys	Asp
	50					55					60				
Val	Ile	Gln	Lys	Gly	Ile	Ser	Val	Val	Gly	Asp	Leu	Leu	Gly	Val	Val
65					70					75					80

Gly 85	Phe 86	Pro 87	Phe 88	Gly 89	Gly 90	Ala 91	Leu 92	Val 93	Ser 94	Phe 95	Tyr 96	Thr 97	Asn 98	Phe 99	Leu 100
Asn 101	Thr 102	Ser 103	Trp 104	Pro 105	Ser 106	Glu 107	Asp 108	Pro 109	Trp 110	Lys 111	Ala 112	Phe 113	Met 114	Glu 115	Gln 116
Val 117	Glu 118	Ala 119	Leu 120	Met 121	Asp 122	Gln 123	Lys 124	Ile 125	Ala 126	Asp 127	Tyr 128	Ala 129	Lys 130	Asn 131	Lys 132
Ala 133	Leu 134	Ala 135	Glu 136	Leu 137	Gln 138	Gly 139	Leu 140	Gln 141	Asn 142	Asn 143	Val 144	Glu 145	Asp 146	Tyr 147	Val 148
Ser 149	Ala 150	Leu 151	Ser 152	Ser 153	Trp 154	Gln 155	Lys 156	Asn 157	Pro 158	Val 159	Ser 160	Ser 161	Arg 162	Asn 163	Pro 164
His 165	Ser 166	Gln 167	Gly 168	Arg 169	Ile 170	Arg 171	Glu 172	Leu 173	Phe 174	Ser 175	Gln 176	Ala 177	Glu 178	Ser 179	Tyr 180
Phe 181	Arg 182	Asn 183	Ser 184	Met 185	Pro 186	Ser 187	Phe 188	Ala 189	Ile 190	Ser 191	Gly 192	Tyr 193	Glu 194	Val 195	Leu 196
Phe 197	Leu 198	Thr 199	Thr 200	Tyr 201	Ala 202	Gln 203	Ala 204	Ala 205	Asn 206	Thr 207	His 208	Leu 209	Phe 210	Leu 211	Leu 212
Lys 213	Asp 214	Ala 215	Gln 216	Ile 217	Tyr 218	Gly 219	Glu 220	Glu 221	Trp 222	Gly 223	Tyr 224	Glu 225	Lys 226	Glu 227	Asp 228
Ile 229	Ala 230	Glu 231	Phe 232	Tyr 233	Lys 234	Arg 235	Gln 236	Leu 237	Lys 238	Leu 239	Thr 240	Gln 241	Glu 242	Tyr 243	Thr 244
Asp 245	His 246	Cys 247	Val 248	Lys 249	Trp 250	Tyr 251	Asn 252	Val 253	Gly 254	Leu 255	Asp 256	Lys 257	Leu 258	Arg 259	Gly 260
Ser 261	Ser 262	Tyr 263	Glu 264	Ser 265	Trp 266	Val 267	Asn 268	Phe 269	Asn 270	Arg 271	Tyr 272	Arg 273	Arg 274	Glu 275	Met 276
Thr 277	Leu 278	Thr 279	Val 280	Leu 281	Asp 282	Leu 283	Ile 284	Ala 285	Leu 286	Phe 287	Pro 288	Leu 289	Tyr 290	Asp 291	Val 292
Arg 293	Leu 294	Tyr 295	Pro 296	Lys 297	Glu 298	Val 299	Lys 300	Thr 301	Glu 302	Leu 303	Thr 304	Arg 305	Asp 306	Val 307	Leu 308
Thr 309	Asp 310	Pro 311	Ile 312	Val 313	Gly 314	Val 315	Asn 316	Asn 317	Leu 318	Arg 319	Gly 320	Tyr 321	Gly 322	Thr 323	Thr 324
Phe 325	Ser 326	Asn 327	Ile 328	Glu 329	Asn 330	Tyr 331	Ile 332	Arg 333	Lys 334	Pro 335	His 336	Leu 337	Phe 338	Asn 339	Tyr 340
Leu 341	Arg 342	Arg 343	Ile 344	Gln 345	Phe 346	His 347	Thr 348	Arg 349	Phe 350	Gln 351	Pro 352	Gly 353	Tyr 354	Tyr 355	Gly 356
Asn 357	Asp 358	Ser 359	Phe 360	Asn 361	Tyr 362	Trp 363	Ser 364	Gly 365	Asn 366	Tyr 367	Val 368	Ser 369	Thr 370	Arg 371	Pro 372
Ser 373	Ile 374	Gly 375	Ser 376	Asn 377	Asp 378	Ile 379	Ile 380	Thr 381	Ser 382	Pro 383	Phe 384	Tyr 385	Gly 386	Asn 387	Lys 388
Ser 389	Ser 390	Glu 391	Pro 392	Val 393	Gln 394	Asn 395	Leu 396	Glu 397	Phe 398	Asn 399	Gly 400	Glu 401	Lys 402	Val 403	Tyr 404
Arg 405	Ala 406	Val 407	Ala 408	Asn 409	Thr 410	Asn 411	Leu 412	Ala 413	Val 414	Trp 415	Pro 416	Ser 417	Ala 418	Val 419	Tyr 420
Ser 421	Gly 422	Val 423	Thr 424	Lys 425	Val 426	Glu 427	Phe 428	Ser 429	Gln 430	Tyr 431	Asn 432	Asp 433	Gln 434	Thr 435	Asp 436
Glu 437	Ala 438	Ser 439	Thr 440	Gln 441	Thr 442	Tyr 443	Asp 444	Ser 445	Lys 446	Arg 447	Asn 448	Val 449	Gly 450	Ala 451	Val 452
Ser 453	Trp 454	Asp 455	Ser 456	Ile 457	Asp 458	Gln 459	Leu 460	Pro 461	Pro 462	Glu 463	Thr 464	Thr 465	Asp 466	Glu 467	Pro 468
Leu 469	Glu 470	Lys 471	Gly 472	Tyr 473	Ser 474										



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500	505	510
Pro Leu Val Lys Ala Tyr Lys	Leu Gln Ser Gly Ala Ser Val Val Ala	
515	520	525
Gly Pro Arg Phe Thr Gly	Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly	
530	535	540
Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys		
545	550	555
Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr		
565	570	575
Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr		
580	585	590
Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser		
595	600	605
Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val		
610	615	620
Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe		
625	630	635
Ile Pro Val Asn		
<210> SEQ ID NO 27		
<211> LENGTH: 644		
<212> TYPE: PRT		
<213> ORGANISM: Bacillus thuringiensis		
<400> SEQUENCE: 27		
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Thr Thr Glu		
1	5	10
Asn Asn Glu Val Pro Thr Asn His Val Gln Tyr Pro Leu Ala Glu Thr		
20	25	30
Pro Asn Pro Thr Leu Glu Asp Leu Asn Tyr Lys Glu Phe Leu Arg Met		
35	40	45
Thr Ala Asp Asn Asn Thr Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp		
50	55	60
Val Ile Gln Lys Gly Ile Ser Val Val Gly Asp Leu Leu Gly Val Val		
65	70	75
Gly Phe Pro Phe Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Phe Leu		
85	90	95
Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Glu Gln		
100	105	110
Val Glu Ala Leu Met Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asn Lys		
115	120	125
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Val Glu Asp Tyr Val		
130	135	140
Ser Ala Leu Ser Ser Trp Gln Lys Asn Pro Val Ser Ser Arg Asn Pro		
145	150	155
His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His		
165	170	175
Phe Arg Asn Ser Met Pro Ser Phe Ala Ile Ser Gly Tyr Glu Val Leu		
180	185	190
Phe Leu Thr Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Phe Leu Leu		
195	200	205
Lys Asp Ala Gln Ile Tyr Gly Glu Glu Trp Gly Tyr Glu Lys Glu Asp		
210	215	220
Ile Ala Glu Phe Tyr Lys Arg Gln Leu Lys Leu Thr Gln Glu Tyr Thr		

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225	230	235	240
Asp His Cys Val Lys Trp Tyr Asn Val Gly Leu Asp Lys Leu Arg Gly	245	250	255
Ser Ser Tyr Glu Ser Trp Val Asn Phe Asn Arg Tyr Arg Arg Glu Met	260	265	270
Thr Leu Thr Val Leu Asp Leu Ile Ala Leu Phe Pro Leu Tyr Asp Val	275	280	285
Arg Leu Tyr Pro Lys Glu Val Lys Thr Glu Leu Thr Arg Asp Val Leu	290	295	300
Thr Asp Pro Ile Val Gly Val Asn Asn Leu Arg Gly Tyr Gly Thr Thr	305	310	315
Phe Ser Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr	325	330	335
Leu His Arg Ile Gln Phe His Thr Arg Phe Gln Pro Gly Tyr Tyr Gly	340	345	350
Asn Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Pro	355	360	365
Ser Ile Gly Ser Asn Asp Ile Ile Thr Ser Pro Phe Tyr Gly Asn Lys	370	375	380
Ser Ser Glu Pro Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Tyr	385	390	395
Arg Ala Val Ala Asn Thr Asn Leu Ala Val Trp Pro Ser Ala Val Tyr	405	410	415
Ser Gly Val Thr Lys Val Glu Phe Ser Gln Tyr Asn Asp Gln Thr Asp	420	425	430
Glu Ala Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val Gly Ala Val	435	440	445
Ser Trp Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro	450	455	460
Leu Glu Lys Gly Tyr Ser His Gln Leu Asn Tyr Val Met Cys Phe Leu	465	470	475
Met Gln Gly Ser Arg Gly Thr Ile Pro Val Leu Thr Trp Thr His Lys	485	490	495
Ser Val Asp Phe Phe Asn Met Ile Asp Ser Lys Lys Ile Thr Gln Leu	500	505	510
Pro Leu Val Lys Ala Tyr Lys Leu Gln Ser Gly Ala Ser Val Val Ala	515	520	525
Gly Pro Arg Phe Thr Gly Gly Asp Ile Ile Gln Cys Thr Glu Asn Gly	530	535	540
Ser Ala Ala Thr Ile Tyr Val Thr Pro Asp Val Ser Tyr Ser Gln Lys	545	550	555
Tyr Arg Ala Arg Ile His Tyr Ala Ser Thr Ser Gln Ile Thr Phe Thr	565	570	575
Leu Ser Leu Asp Gly Ala Pro Phe Asn Gln Tyr Tyr Phe Asp Lys Thr	580	585	590
Ile Asn Lys Gly Asp Thr Leu Thr Tyr Asn Ser Phe Asn Leu Ala Ser	595	600	605
Phe Ser Thr Pro Phe Glu Leu Ser Gly Asn Asn Leu Gln Ile Gly Val	610	615	620
Thr Gly Leu Ser Ala Gly Asp Lys Val Tyr Ile Asp Lys Ile Glu Phe	625	630	635
Ile Pro Val Asn			640

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<210> SEQ ID NO 28
<211> LENGTH: 651
<212> TYPE: PRT
<213> ORGANISM: Bacillus thuringiensis

<400> SEQUENCE: 28

Met Asn Pro Asn Asn Arg Ser Glu Tyr Asp Thr Ile Lys Val Thr Pro
 1             5             10             15

Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
 20             25             30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
 35             40             45

Thr Ala Asp Asn Ser Thr Glu Val Leu Asp Ser Ser Thr Val Lys Asp
 50             55             60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
 65             70             75             80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
 85             90             95

Asn Ala Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100            105            110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115            120            125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130            135            140

Val Asn Ala Leu Asp Ser Trp Lys Lys Ala Pro Val Asn Leu Arg Ser
145            150            155            160

Arg Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165            170            175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180            185            190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195            200            205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210            215            220

Asp Ile Ala Glu Phe Tyr Gln Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225            230            235            240

Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Ser Leu Arg
245            250            255

Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
260            265            270

Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
275            280            285

Val Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
290            295            300

Phe Thr Asp Pro Ile Phe Thr Leu Asn Ala Leu Gln Glu Tyr Gly Pro
305            310            315            320

Thr Phe Ser Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
325            330            335

Tyr Leu Arg Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr Ser
340            345            350

Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
355            360            365

Pro Ser Ile Gly Ser Asn Asp Thr Ile Thr Ser Pro Phe Tyr Gly Asp
370            375            380

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Lys Ser Ile Glu Pro Ile Gln Lys Leu Ser Phe Asp Gly Gln Lys Val  
 385 390 395 400  
 Tyr Arg Thr Ile Ala Asn Thr Asp Ile Ala Ala Phe Pro Asp Gly Lys  
 405 410 415  
 Ile Tyr Phe Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln  
 420 425 430  
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Tyr Asn Gly  
 435 440 445  
 Tyr Leu Gly Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr  
 450 455 460  
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu  
 465 470 475 480  
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp  
 485 490 495  
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile  
 500 505 510  
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser  
 515 520 525  
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys  
 530 535 540  
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala  
 545 550 555 560  
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr  
 565 570 575  
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile  
 580 585 590  
 Tyr Ile Asn Lys Thr Met Asn Ile Asp Gly Asp Leu Thr Tyr Gln Thr  
 595 600 605  
 Phe Asp Phe Ala Thr Ser Asn Ser Asn Met Gly Phe Ser Gly Asp Thr  
 610 615 620  
 Asn Asp Phe Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile  
 625 630 635 640  
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln  
 645 650

&lt;210&gt; SEQ ID NO 29

&lt;211&gt; LENGTH: 651

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 29

Met Asn Pro Asn Asn Arg Ser Glu Tyr Asp Thr Ile Lys Val Thr Pro  
 1 5 10 15  
 Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn  
 20 25 30  
 Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met  
 35 40 45  
 Thr Ala Asp Asn Ser Thr Glu Val Leu Asp Ser Ser Thr Val Lys Asp  
 50 55 60  
 Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val  
 65 70 75 80  
 Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu  
 85 90 95  
 Asn Ala Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala

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100						105						110				
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	
		115					120					125				
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	
	130					135					140					
Val	Asn	Ala	Leu	Asp	Ser	Trp	Lys	Lys	Ala	Pro	Val	Asn	Leu	Arg	Ser	
145					150					155					160	
Arg	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	
				165					170					175		
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	
			180					185					190			
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	
		195					200					205				
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	
	210					215					220					
Asp	Ile	Ala	Glu	Phe	Tyr	Gln	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	
225					230					235					240	
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Ser	Leu	Arg	
				245					250					255		
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
			260						265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	
		275					280						285			
Val	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
	290					295					300					
Phe	Thr	Asp	Pro	Ile	Phe	Thr	Leu	Asn	Ala	Leu	Gln	Glu	Tyr	Gly	Pro	
305					310					315					320	
Thr	Phe	Ser	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
				325					330					335		
Tyr	Leu	Arg	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Arg	Pro	Gly	Tyr	Ser	
			340					345						350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	
		355					360						365			
Pro	Ser	Ile	Gly	Ser	Asn	Asp	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	
	370					375										
Lys	Ser	Ile	Glu	Pro	Ile	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	
385					390					395					400	
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Ile	Ala	Ala	Phe	Pro	Asp	Gly	Lys	
				405					410						415	
Ile	Tyr	Phe	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	
			420					425						430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Tyr	Asn	Gly	
		435					440						445			
Tyr	Leu	Gly	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	
	450					455						460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	
465					470					475					480	
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	
				485					490					495		
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	
			500					505					510			
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	
		515					520						525			

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Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
530                               535                               540

Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
545                               550                               555                               560

Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
                               565                               570                               575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
                               580                               585                               590

Tyr Ile Asn Lys Thr Met Asn Ile Asp Gly Asp Leu Thr Tyr Gln Thr
                               595                               600                               605

Phe Asp Phe Ala Thr Ser Asn Ser Asn Met Gly Phe Ser Gly Asp Thr
610                               615                               620

Asn Asp Phe Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625                               630                               635                               640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln
                               645                               650

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<210> SEQ ID NO 30
<211> LENGTH: 652
<212> TYPE: PRT
<213> ORGANISM: Bacillus thuringiensis

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<400> SEQUENCE: 30

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1                               5                               10                               15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20                               25                               30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35                               40                               45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50                               55                               60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65                               70                               75                               80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85                               90                               95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100                              105                              110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115                              120                              125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130                              135                              140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145                              150                              155                              160

Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165                              170                              175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180                              185                              190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195                              200                              205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210                              215                              220

Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225                              230                              235                              240

Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg

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245								250				255			
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
			260					265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290					295					300				
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
				325					330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
			340					345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
		355					360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
	370					375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
			420					425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
		435					440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450					455					460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465					470					475					480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
				485					490					495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
			500					505					510		
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
		515					520					525			
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
	530					535					540				
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
545					550					555					560
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
				565					570					575	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile
			580					585					590		
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
		595					600					605			
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys
	610					615					620				
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile
625					630					635					640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				
				645						650					

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<211> LENGTH: 652
<212> TYPE: PRT
<213> ORGANISM: Bacillus thuringiensis

<400> SEQUENCE: 31
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
 1             5             10             15
Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
 20             25             30
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
 35             40             45
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
 50             55             60
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
 65             70             75             80
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
 85             90             95
Asp Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
 100            105            110
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
 115            120            125
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
 130            135            140
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
 145            150            155            160
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
 165            170            175
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
 180            185            190
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
 195            200            205
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
 210            215            220
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
 225            230            235            240
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
 245            250            255
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
 260            265            270
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
 275            280            285
Val Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
 290            295            300
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro
 305            310            315            320
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
 325            330            335
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Ser
 340            345            350
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
 355            360            365
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370            375            380
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val

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385	390	395	400
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys			
	405	410	415
Ile Tyr Phe Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln			
	420	425	430
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly			
	435	440	445
His Val Gly Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr			
	450	455	460
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu			
	465	470	475
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp			
	485	490	495
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile			
	500	505	510
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser			
	515	520	525
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys			
	530	535	540
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala			
	545	550	555
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr			
	565	570	575
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Ile Val Ile			
	580	585	590
Tyr Ile Asn Lys Thr Met Asn Ile Asp Asp Asp Leu Thr Tyr Gln Thr			
	595	600	605
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Thr			
	610	615	620
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile			
	625	630	635
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu			
	645	650	

&lt;210&gt; SEQ ID NO 32

&lt;211&gt; LENGTH: 347

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 32

Lys Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro Thr			
1	5	10	15
Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp Tyr			
	20	25	30
Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe Gly			
	35	40	45
Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg Pro			
	50	55	60
Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp Lys			
	65	70	75
Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr			
	85	90	95
Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys Val			
	100	105	110

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Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln Lys  
 115 120 125  
 Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly His  
 130 135 140  
 Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp  
 145 150 155 160  
 Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys  
 165 170 175  
 Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp Thr  
 180 185 190  
 His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile Thr  
 195 200 205  
 Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile  
 210 215 220  
 Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu  
 225 230 235 240  
 Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala Ala  
 245 250 255  
 Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn  
 260 265 270  
 Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr  
 275 280 285  
 Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe  
 290 295 300  
 Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys Asn  
 305 310 315 320  
 Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr  
 325 330 335  
 Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu  
 340 345

&lt;210&gt; SEQ ID NO 33

&lt;211&gt; LENGTH: 649

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus thuringiensis

&lt;400&gt; SEQUENCE: 33

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Ala Thr Glu  
 1 5 10 15  
 Asn Asn Glu Val Ser Asn Asn His Ala Gln Tyr Pro Leu Ala Asp Thr  
 20 25 30  
 Pro Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Arg Thr Thr  
 35 40 45  
 Asp Asn Asn Val Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp Ala Ile  
 50 55 60  
 Gln Lys Gly Ile Ser Ile Ile Gly Asp Leu Leu Gly Val Val Gly Phe  
 65 70 75 80  
 Pro Tyr Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Leu Leu Asn Thr  
 85 90 95  
 Ile Trp Pro Gly Glu Asp Pro Leu Lys Ala Phe Met Gln Gln Val Glu  
 100 105 110  
 Ala Leu Ile Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asp Lys Ala Thr  
 115 120 125  
 Ala Glu Leu Gln Gly Leu Lys Asn Val Phe Lys Asp Tyr Val Ser Ala  
 130 135 140

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Leu Asp Ser Trp Asp Lys Thr Pro Leu Thr Leu Arg Asp Gly Arg Ser															
145				150					155						160
Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His Phe Arg															
			165					170						175	
Arg Ser Met Pro Ser Phe Ala Val Ser Gly Tyr Glu Val Leu Phe Leu															
			180					185						190	
Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu Lys Asp															
			195					200						205	
Ala Gln Ile Tyr Gly Thr Asp Trp Gly Tyr Ser Thr Asp Asp Leu Asn															
			210					215					220		
Glu Phe His Thr Lys Gln Lys Asp Leu Thr Ile Glu Tyr Thr Asn His															
			225					230					235		240
Cys Ala Lys Trp Tyr Lys Ala Gly Leu Asp Lys Leu Arg Gly Ser Thr															
			245						250					255	
Tyr Glu Glu Trp Val Lys Phe Asn Arg Tyr Arg Arg Glu Met Thr Leu															
			260						265					270	
Thr Val Leu Asp Leu Ile Thr Leu Phe Pro Leu Tyr Asp Val Arg Thr															
			275						280				285		
Tyr Thr Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Val Leu Thr Asp															
			290					295					300		
Pro Ile Val Ala Val Asn Asn Met Asn Gly Tyr Gly Thr Thr Phe Ser															
			305					310					315		320
Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr Leu His															
			325						330					335	
Ala Ile Gln Phe His Ser Arg Leu Gln Pro Gly Tyr Phe Gly Thr Asp															
			340						345				350		
Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Ser Ser Ile															
			355						360				365		
Gly Ser Asp Glu Ile Ile Arg Ser Pro Phe Tyr Gly Asn Lys Ser Thr															
			370						375				380		
Leu Asp Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Phe Arg Ala															
			385						390				395		400
Val Ala Asn Gly Asn Leu Ala Val Trp Pro Val Gly Thr Gly Gly Thr															
			405						410					415	
Lys Ile His Ser Gly Val Thr Lys Val Gln Phe Ser Gln Tyr Asn Asp															
			420						425					430	
Arg Lys Asp Glu Val Arg Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val															
			435						440				445		
Gly Gly Ile Val Phe Asp Ser Ile Asp Gln Leu Pro Pro Ile Thr Thr															
			450						455				460		
Asp Glu Ser Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Val Arg															
			465						470				475		480
Cys Phe Leu Leu Gln Gly Gly Arg Gly Ile Ile Pro Val Phe Thr Trp															
			485						490					495	
Thr His Lys Ser Val Asp Phe Tyr Asn Thr Leu Asp Ser Glu Lys Ile															
			500						505					510	
Thr Gln Ile Pro Phe Val Lys Ala Phe Ile Leu Val Asn Ser Thr Ser															
			515						520					525	
Val Val Ala Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Lys Cys Thr															
			530						535					540	
Asn Gly Ser Gly Leu Thr Leu Tyr Val Thr Pro Ala Pro Asp Leu Thr															
			545						550				555		560

-continued

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Tyr	Ser	Lys	Thr	Tyr	Lys	Ile	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Ser	Gln
				565					570					575	
Val	Arg	Phe	Gly	Ile	Asp	Leu	Gly	Ser	Tyr	Thr	His	Ser	Ile	Ser	Tyr
			580					585					590		
Phe	Asp	Lys	Thr	Met	Asp	Lys	Gly	Asn	Thr	Leu	Thr	Tyr	Asn	Ser	Phe
		595					600					605			
Asn	Leu	Ser	Ser	Val	Ser	Arg	Pro	Ile	Glu	Ile	Ser	Gly	Gly	Asn	Lys
	610					615					620				
Ile	Gly	Val	Ser	Val	Gly	Gly	Ile	Gly	Ser	Gly	Asp	Glu	Val	Tyr	Ile
625					630				635						640
Asp	Lys	Ile	Glu	Phe	Ile	Pro	Met	Asp							
				645											

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That which is claimed:

1. A modified Cry3 pesticidal polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 8, 11, or 14.

2. A polynucleotide having a nucleotide sequence encoding the modified Cry3 pesticidal polypeptide of claim 1.

3. The polynucleotide of claim 2, wherein said nucleotide sequence is set forth in SEQ ID NO: 7, 9, 10, 12, 13, or 15.

4. An expression cassette comprising the polynucleotide of claim 2.

5. The expression cassette of claim 4, wherein said polynucleotide is operably linked to a promoter that drives expression in a microorganism or a plant.

6. A host cell comprising the polynucleotide of claim 2.

7. A plant comprising the polynucleotide of claim 2, wherein said polynucleotide is operably linked to a promoter active in said plant.

8. The plant of claim 7, wherein said plant is a monocotyledonous plant.

9. The plant of claim 7, wherein said plant is a dicotyledonous plant.

10. The plant of claim 8, wherein said monocotyledonous plant is selected from the group consisting of maize, sugarcane, wheat, rice, barley, sorghum, and rye.

11. The plant of claim 10, wherein said monocotyledonous plant is maize.

12. A transgenic seed produced by the plant of claim 7.

13. A method for producing a plant having improved pesticidal activity, said method comprising introducing into said plant a polynucleotide having a nucleotide sequence that encodes a modified Cry3 pesticidal polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 8, 11, or 14.

14. A pesticidal composition comprising the modified Cry3 pesticidal polypeptide of claim 1.

15. The pesticidal composition of claim 14, further comprising a carrier.

16. A method for controlling an insect pest in an area of cultivation comprising applying an effective amount of the pesticidal composition of claim 14 to an environment of said insect pest.

17. The method of claim 16, wherein said insect pest is a Coleopteran.

18. An expression cassette comprising the polynucleotide of claim 3.

19. The expression cassette of claim 18, wherein said polynucleotide is operably linked to a promoter that drives expression in a microorganism or a plant.

20. A host cell comprising the polynucleotide of claim 3.

21. A plant comprising the polynucleotide of claim 3, wherein said polynucleotide is operably linked to a promoter active in said plant.

22. The plant of claim 21, wherein said plant is a monocotyledonous plant.

23. The plant of claim 21, wherein said plant is a dicotyledonous plant.

24. The plant of claim 22, wherein said monocotyledonous plant is selected from the group consisting of maize, sugarcane, wheat, rice, barley, sorghum, and rye.

25. The plant of claim 24, wherein said monocotyledonous plant is maize.

26. A transgenic seed produced by the plant of claim 21 comprising said polynucleotide.

\* \* \* \* \*